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(71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(72) Inventors; and

- (75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J. [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). TONER, Timothy, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.





For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

REFERENCE TO MICROFICHE APPENDIX

15 Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant-invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

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Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The nef gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The env gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

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The tat gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The rev gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

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Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8+T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

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European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including env or gag. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 J. Virol. 64(5):2047-2056; Gräble and Hearing, 1992 J. Virol. 66(2):723-731.

Larder, et al., (1987, Nature 327: 716-717) and Larder, et al., (1989, Proc. Natl. Acad. Sci. 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on in vitro activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HTV-1 Pol.

Schatz, et al. (1989, FEBS Lett. 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, J. Biol. Chem. 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, J. Virol. 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

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The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or 15 HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human 20 mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of 25 HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression 30 of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized 35 versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

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The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

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Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

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In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

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The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

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It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

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It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors. It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to - highly active antiretroviral therapy -.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

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"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

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In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

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"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BglII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

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Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

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Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

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Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEO ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

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Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+γIFN+ and CD4+γIFN+, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

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DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

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A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

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As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities in vitro when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice in vivo with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

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In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized env sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

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A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at 10 least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International 15 Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an 20 amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at 25 the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first 30 generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID 35 NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

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The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate 10 studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-15 nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and 20 PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein 25 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef 30 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and 35 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

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described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMVnef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

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Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with 30 one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the 35 MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

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The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

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Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

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Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle 20 plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 25 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by 30 reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As 35 examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g.,, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

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Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

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The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" Advances in Pharmacology 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6® cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®], from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 J. Gen. Virol 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl₂; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl₂, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of 1×10^{7} to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

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This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

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EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter GMP grade pVIInsHIVgag was used as the starting material to amplify the hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Msc1 site of the hCMV promoter and a 3' primer (designed to contain the Bg/III recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Taq polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and BgIII. This fragment was then cloned back into the original GMP grade pV1InsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and BgIII digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using BgIII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BgIII site. Colonies were screened using Sma1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

<u>AATAAA</u>AGATCTTTATTTTCATTAGATCT<u>GTGTG TTGGTTTTTTGTGTG</u> (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

15 EXAMPLE 2

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Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901ª	10.8
PVIIns-hCMV-FLgag-bGHpAb	16.6
pV1Ins-hCMV-FLgag-SPAbc	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

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EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes
A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 µg and 200 µg.

b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA®	Dose,		Anti-p24 Titers (3 Wk PD1) ^c			SFC/10^6 Cells (4 Wk PD1) ^d			
Promoter/terminator		GMT	+\$E	-SE	Media	gag197-205	p24		
HIVFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)		
(GMP grade)	20	5572	1574	1227	0	56(9)	25(6)		
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)		
FL-gag-bGHpA	20	7352	2808	2032	0	73(9)	11(6)		
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)		
FL-gag-SPA	20	5971	5361	2825	0	85(17)	38(10)		
Naīve	0	123	50	36	0	0	0		

in PBS

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Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6[®] cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 µL per quad

[°]n=10;GMT, geometric mean titer; SE, standard. error

dn=5, pooled spleens; mean of triplicate wells and standard, deviation, in parentheses;

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 10 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction 15 digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple 20 cloning site of the original shuttle vector contained ClaI, BarnHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made 25 to the packaging region and pIX gene.

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

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and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

EXAMPLE 7

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Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with HindIII and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with Msc1 overnight and then digested with Sfi1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

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EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with Pac1. The reaction mixture was digested with BsfZ171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with Cla1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into E. coli BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml TerrificTM broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 μl dH₂0. A 2 μl aliquot of this DNA was transformed into E. coli XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme BstEII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

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EXAMPLE 11

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6® cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pac1/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

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EXAMPLE 12

Stability Analyses

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To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses 20 from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgagbGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

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Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4: Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio			
MRKAd5gag	470			
HCMV-Flgag-bGHpA [E3-]	115			
HCMV-Flgag-SPA [E3+]	320			
mCMV-FLgag-bGHpA [E3+]	420			
Original construct *	40 - 50			

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EXAMPLE 13

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Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

^{*} This estimation is based on the clinical lot growth characteristics at Passage 12.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

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Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	Xv (10° calls/m	i), Viablety (%) Harvest	Harvest Time	Coll Passage Number	Titler 10" vp/mi culture	Ther 10" vp/cell	OPA 10° TCID ₈₀ /ml	Ratio AEX:OPA	Amplification Ratio	AEX Internal Contro
P4	1,49, 81%	0.58, 50%	44	45	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 93%	0.66, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1,04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	į
P7	1,50, 84%	0.95, 61%	49.5	50	3.9	1,4	0.97	40	50	İ
P7	1.09, 97%	0.76, 69%	50	52	5.2	4.7	1.70	81	170	İ
P8	1.03, 94%	0.85, 84%	47.5	54	9.0	8.7	1.10	82	310	
P9	0.89, 95%	0.99, 73%	47.5	56	4,4	4.9	1.03	43	175	3.12 2.84
P10	1.09, 91%	1,06, 66%	47.5	58	3.0	2,8	1.16	26	100	2.70 2.60
P11	1,19,58%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0,58, 91%	0.85, 63%	47,5	47	5.4	5.5	1.20	45	200	2.86 2.60
P13	1,00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
	1,94, 92%	0.88, 57%	46	53	8.6	4.4	 -	 	160	3.28 3.27
P14	" _	0.64, 66%	47	47	6.8	7.1	 	 	250	3.12
P15	0.97, 96%	0.04, 50%	1 "	1 "	1	1	1	1	1	2.91

Table 5B: Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10° cells/m	n, Viability (%)	Harvest Time	Cell Passage	Ther	Titer	QPA	Ratio	Amplification Ratio	AEX Internal Contro
	Insection	Harvest_	hp.L	Number	10 ¹⁰ vp/ml culture	10° vp/cet	10° TCID _{eo} /mil	AEX:QPA	300	TIESTE COMO
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.6	1.70	25	(MOI = 125)	
P5	0.92, 89%	1.18, 77%	47	48	4.3	4.7	1.24	35	170	
P6	1,55, 88%	1.24, 76%	49.5	50	1.2	0.8	0.56	21	30	
P6	1,09, 97%	1,11, 81%	49	52	4.0	3.6	1.16	94	130	1
	1,17, 91%	1.22.91%	47.5	54	3.7	3.2	0.50	74	110	1
P7	1.37, 9170	1.22, 51, 7				L	<u> </u>	45	75	3.12
P8	0.98, 88%	1.41, 83%	4B	56	2.1	21	0.47			2.84
P9	1.20, 89%	1,28, 81%	47.5	58	0.8	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.55, 85%	47	60	23	23	0.43	83	80	2.70 2.70
	1.07. 96%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.86
P11	1,07, 90%	1.23, 23,				1		123	250	2.60 3.18
P12	0.80, 91%	1.14, 80%	49,5	49	6.9	7.4	0.48	123		3.18
P13	1.96, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.26 3.27
P14	0.87, 96%	1.03, 98%	48.5	47	B.4	9.7	T	T	350	3.12 2.91
	1	L	49,5	49	5.3	6.1	+	 	218	2.78
P15	0.87, 99%	0.97, 59%	49.5	1 "	1	1		1 .	1	2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

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MRKAd5gag(E3-)

	Xv (10° cells/n	n), Vieblity (%) Harvest	Harvest Time h.p.l.	Cell Passage Number	Titer 10 ¹⁹ vp/ml culture	Titer 10° vp/ceil	QPA 10° TCID _{EO} /mi	REID AEX: GPA	Amplification Railo	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.52	20	100 (MOI=125)	HARMED COMMON
P5	1.16, 92%	0.52, 43%	49	49	3.3	2.0	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
PB	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 68%	0.77, 48%	48	56	3.1	3.2	0.68	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	63.0	47	115	2.70 2.70
P11	1.07, 95%	0.98, 70%	48.5	47	5.9	5.5	0.68	B7	200	2.88 2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6.A	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7,4	3.8			185	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 99%	0.84, 56%	49	49	4,8	5.5			196	2.78 2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (107 and 109 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

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Viral Vectors	μg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gagd	1.32
MCMVFL-gagbGHpA ^e	0.42

^a A_{260nm} absorbance readings taken for viral particle determinations.

b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

²⁵ d Research Ad5FLgag lot# 6399

[°] mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
	SADVA JEnor	10^7	25600	5877	4780
1 2	^a MRKAd5gag "	10^9	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
4	nominate gas an appropriate to provide the second s	10^9	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
5 6	•	10^9	310419	99181	75165
7	bmCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
8		10^9	941014	239068	190636
9	^c hCMV FL-gag bGHpA [E3-] ←	10^7	3676	934	745
10		10^9	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
12		10^7 10^8	14703 58813	5274 14942	3882 11915
13 14	•	10/9	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
16		10^7	4222	3405	1138
17	•	10^8	19401	3939	3274
18	P	10^9	89144	25187	19639
19	Naĭve	none	93	7	6

*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro Vaccination: T. Toner, Q. Su

Assay: M. Chen

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 a The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] ightarrow The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10^{11} vp and 10^9 vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

The same lot of mCMVFL-gagbGHpA[E3+] used in the in vitro study (Table 6) ws used here.

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

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peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

ag-expressing adenovecto	Pre	Wk4	Wk8	Wk 12	Wk 16_	Wk 20	Wk 25	Wk 28
/IRKAd5gag°, 10^11 vp	<10	118	5528	11523	7062	21997	ND	51593
7N010	<10	62	772	1447	1562	2174	ND	20029
77N116		66.	3353	6156	6845	3719	ND	24031
98X007	<10	- 00:	3000	0.00				
VIRK Ad5gog, 10^9 vp				018	366	482	ND	6550
77N120	<10	51	204	318	706	888	ND	7136
97N144	<10	18	118	274		1072	ND	12851
98X008	<10	15	444	386_	996	10/2	ND	12001
Ad5gag ^b , Clinical Lat, 10^11 vp					7174	7250	ND	69226
97X001	<10	87	2579	4718	7174		ND	60283
97N146	<10_	72	3604_	7380	7526	18906		26226
98X009	<10	78	4183	3946	3124	6956	ND_	20220
Ad5gag, Clinical Lat, 10^9 vp						1001	NE	17177
97N020	<10	<10	143_	371	390	1821	ND_	
97X003	<10	<10	39	93	156	596	ND_	2053
98X012	<10	81	342	717	956	1558	ND_	11861
SARK ACTIONS (DCMV, bGHpA, E3+			<u> </u>	 		 	+	+
bariginal Adagog vector (hCMV/Intro	on A. bGHp	A, E3-), id	#FN0001					
ND, not determined			<u> </u>					

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Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Gno #	Vaccination	Monkey ID	1=4	Wk	Î pê	Wk	[គ]	Wk	Isl.	5 Wk	T ∓2	Wk_	In2	Wk
G.P.	T=0,4,25 wks		Media	Gog H ^b	Media	Gog H	Medio	Gag H	Medic	Gog H	Media	Goo H	Media	Gog H
1	MRKAc6gog	97ND1D 97ND10(CD4-)	6 4	89 38	0	395	0 3	1058 993	0	1174	3	775 76	4	1074 594
	10^11 vp	97N116	1	398	1	609	Ó	534	4	395	ĩ	261	Ŏ	408
		97N116(CD4-)	11	676			0	593 2193	1	2118	3	184 1588	8	666 2113
		98X007 98X007(CCC4-)	10 20	579 965	0	1304	3 0	2675	'	2118	ő	1656	Ö	1278
2	MRKAc5000 10/9 VD	97N120 97N120(CD4-)	5 11	275. 170	1	249	4 0	141 85	4	119	ô	206 75	4	219 219
	ID-3 Ab	97N144 97N144(CD4-)	3 6	236 148	6	438	ì	318 285	3	256	ND ND	98 OM	5	373 625
		98X008 98X008(CD4-)	14	S68 696	1	1090	3	891 1175	4	673	3	473 391	5	735 848
				261	-	485	0	B17	0	1220b	<u> </u>	894	0	1858
3 .	Actigacy clinical lat 10^11 vp	97X001 97X001(CD4-)	10 3	283 150	',	465	3	996 339	,	1272	ģ	1010	0 3	1123
		97N146 97N146(CD4-)	6	133	l '		Ō	370			Ö	654 384	Ö	971 1748
		98X009 98X009(CD4-)	Ô	93 73	3	339	3 0	559 333	°	896	ó	225	ő	644
4	AdSigna dinical lat 10/9 vp	97N020 97N020(CD4-)	3 10	30 29	1	101	0	66 15	0	36 38	0	26 1 38	00	41 16 81
		97X003 97X003(CD4-)	9	68 40 95	5	134	0	18 6 34		18	0	4 20	ļ	19
		98X012 98X012(CD4-)	5 11	70			Ö	ii	١	"	ŏ	8	ò	41
5	Nave	96R041 053F	B 14	8 18	1 5	1 16	20	0 14	19	0 15	0 10	0 15	24	00

Based on either 4x10/6 or 2x10/6 cells per well (depending on spot density)

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The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses in vivo even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

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⁵ Pad of 20-corporates overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing the corporate overlapping by 10 corandencompositing by 10 cor

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on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred 10 ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr 15 (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization 20 or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a 25 preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wtpol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows: AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

	GAAATCTGCA	CTGAGATGGA	GAAGGAGGC	AAAATCTCCA	AGATTGGCCC	CGAGAACCCC
	TACAACACCC	CTGTGTTTGC	CATCAAGAAG	AAGGACTCCA	CCAAGTGGAG	GAAGCTGGTG
	GACTTCAGGG	AGCTGAACAA	GAGGACCCAG	GACTTCTGGG	AGGTGCAGCT	GGGCATCCCC
	CACCCCGCTG	GCCTGAAGAA	GAAGAAGTCT	GTGACTGTGC	TGGATGTGGG	GGATGCCTAC
5	TTCTCTGTGC	CCCTGGATGA	GGACTTCAGG	AAGTACACTG	CCTTCACCAT	CCCCTCCATC
	AACAATGAGA	CCCCTGGCAT	CAGGTACCAG	TACAATGTGC	TGCCCCAGGG	CTGGAAGGGC
	TCCCCTGCCA	TCTTCCAGTC	CTCCATGACC	AAGATCCTGG	AGCCCTTCAG	GAAGCAGAAC
	CCTGACATTG	TGATCTACCA	GTACATGGAT	GACCTGTATG	TGGGCTCTGA	CCTGGAGATT
	GGGCAGCACA	GGACCAAGAT	TGAGGAGCTG	AGGCAGCACC	TGCTGAGGTG	GGGCCTGACC
10	ACCCCTGACA	AGAAGCACCA	GAAGGAGCCC	CCCTTCCTGT	GGATGGGCTA	TGAGCTGCAC
	CCCGACAAGT	GGACTGTGCA	GCCCATTGTG	CTGCCTGAGA	AGGACTCCTG	GACTGTGAAT
	GACATCCAGA	AGCTGGTGGG	CAAGCTGAAC	TGGGCCTCCC	AAATCTACCC	TGGCATCAAG
	GTGAGGCAGC	TGTGCAAGCT	GCTGAGGGGC	ACCAAGGCCC	TGACTGAGGT	GATCCCCCTG
	ACTGAGGAGG	CTGAGCTGGA	GCTGGCTGAG	AACAGGGAGA	TCCTGAAGGA	GCCTGTGCAT
15	GGGGTGTACT	ATGACCCCTC	CAAGGACCTG	ATTGCTGAGA	TCCAGAAGCA	GGGCCAGGGC
	CAGTGGACCT	ACCAAATCTA	CCAGGAGCCC	TTCAAGAACC	TGAAGACTGG	CAAGTATGCC
	AGGATGAGGG	GGGCCCACAC	CAATGATGTG	AAGCAGCTGA	CTGAGGCTGT	GCAGAAGATC
	ACCACTGAGT	CCATTGTGAT	CTGGGGCAAG	ACCCCCAAGT	TCAAGCTGCC	CATCCAGAAG
	GAGACCTGGG	AGACCTGGTG	GACTGAGTAC	TGGCAGGCCA	CCTGGATCCC	TGAGTGGGAG
20	TTTGTGAACA	CCCCCCCCT	GGTGAAGCTG	TGGTACCAGC	TGGAGAAGGA	GCCCATTGTG
		CCTTCTATGT				
		CCAACAGGGG				
		TCCAGGCCAT				
	GTGACTGACT	CCCAGTATGC	CCTGGGCATC	ATCCAGGCCC	AGCCTGATCA	GTCTGAGTCT
25		ACCAGATCAT				
		ACAAGGGCAT	•			
	ATCAGGAAGG	TGCTGTTCCT	GGATGGCATT	GACAAGGCCC	AGGATGAGCA	TGAGAAGTAC
	· -	GGAGGGCTAT				
		CCTGTGACAA				
30	TGCTCCCCTG	GCATCTGGCA	GCTGGACTGC	ACCCACCTGG	AGGGCAAGGT	GATCCTGGTG
	GCTGTGCATG	TGGCCTCCGG	CTACATTGAG	GCTGAGGTGA	TCCCTGCTGA	GACAGGCCAG
						CATCCACACT
						GGCTGGCATC
						GTCCATGAAC
35						GAAGACAGCT
	GTGCAGATGG	CTGTGTTCAT	CCACAACTTC	AAGAGGAAGG	GGGGCATCGG	GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGCC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly 20 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile 25 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys 30 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu 35 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile 5 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys 10 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lvs Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val 15 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr 20 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp 25 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

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DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct 5 contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at 10 least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point 15 mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any 20 combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue. 25

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		10010 1		
	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp .	445	. Ala	. RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
	-	678	Ala	IN
	Asp Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

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AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

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ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC TGCTCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG CCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID NO:3).

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In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase 25 function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and 30 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys 10 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr 15 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp 20 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys 30 . Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His 35 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Gly Ile Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Arg Asp Ser Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Gly Glu Gly Ala Val Val Ile Gln Asp Asp Ser Asp Ile Lys Val Asp Ile Ile Arg Asp Asn Ser Asp Ile Lys Val Val Val Ile Gln Asp Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Ala Lys Ile Gln Asp Asp Ser As

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

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To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGT CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT 10 CCCTGAGTGG GAGTTTGTGA ACACCCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA 15 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA 20 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT 25 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT 30 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly

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Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu 5 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 30 Thr Asp Thr Thr Asn Gln Lys Thr.Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp 35 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 5 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 10 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) 20 which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, 25 any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a 30 leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially 35 abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEO ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a 10 "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows: GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT 15 CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC 20 CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG 25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC 10 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT 15 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC 20 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Ile Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu 10 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr 15 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala 20 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile 25 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 30 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 35 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Lleu Leu Trp Lys Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

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Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

PCT/US01/28861 WO 02/022080

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

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The nucleotide sequence of the codon optimized version of HIV-1 jrfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein: GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT ACACCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (ifrl) protein is disclosed herein as SEQ ID 35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Sap Arg Val Arg Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Trp Leu Glu Ala Glu Asp Glu Glu Val Gly Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Ala Asp Clu Glu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Gly Tyr Phe Pro Asp Trp Gln Asp Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Gly Ile Arg Pho Met Ser Gln Lys Val Gly Ala Asp Pro Glu Lys Gly Gly Fro Gly Pro 10

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HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

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Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCCCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTG TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACCCCATGTC
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEO ID NO:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human.

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In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG

5 GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC
CCAAGCTGGC CTTCCACCAC GAGGACCCCG AGAAGAGGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val 15 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp 20 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His 25 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

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An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG

5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT

10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC

15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu 25 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu 30 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a 35 deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

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EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with BgI II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

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Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μ g of pMRKAd5pol was digested with restriction enzyme PacI (New England Biolabs) and 3.3 μ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). PacI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

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MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the Bgl11 site. The clones were checked for correction orientation of the gene by using restriction enzyme Scal. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme *Pac1* (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6 $^{\circ}$ cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60 $^{\circ}$ C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

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Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the Bgl II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the El parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with $Bgl \ II$ and the gag reporter gene ($Bgl \ II$ fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

 $Bgl \ \Pi$ site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Ins plasmids by $Bgl \ \Pi$ digestion.

EXAMPLE 22

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Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac1* and *BstZ110I* digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla* I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BamHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 µL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

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Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 μg/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO4 per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFγ-10 secreting cells from mouse spleens (Miyahira, et al. 1995, J. Immunol. Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5x106/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β -ME). Rhesus PBMCs were prepared from 8-15 15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 µL/well of either 5 µg/mL purified rat anti-mouse IFN-y IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN-γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, 20 Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples (4-5x10⁵ cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

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by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10^{^7} vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

PCT/US01/28861 WO 02/022080

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

'able	10. Immunogenicity	OT IATE	777.07	701 V 0010	I-RT IgG Titer	e.	SFC/10^6 cells*			
	Vaccine	Dasa	No. of Doses	GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool	
Group 1	MRKAd5hCMVFLpol (E3+)	10^7 vp	2	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)	
2	MRKAdShCMVFLpol (E3+)	10^9 vp	2 1	1638400 ⁵ 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2063(182 733(89)	
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2 1	310419 6400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2807 (27) 409 (28)	
4	MRKAd5hCMVFLpol (E3-)	10^9 vp	2	1838400 ^b 1241675 ^b	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)	
	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)	

^{*}GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

able	 Immunogenicit 	y U1 1411		A	ti-ne1 lgG Tite	TS.	SI	FC/10^6 cells	
		Dose	No. of	GMT	+SE	-SE	Medium	2251-70 CD8+	2281-100 CD4+
quora	Vaccine		Doses 2	174	70	50	1(1)	23(1)	1(1)
1	MRKAd5hCMVFLnef (E3+)	10^7 vp	1	132	42	32	0(0)	0(0)	0(0)
ļ				474	70	50	0(0)	61(7)	4(2)
2	MRKAd5hCMVFLnef (ES+)	10^9 vp	2	174 132	42	32	1(1)	62(7)	3(1)
1		10^7 VP	2	132	42	32	3(1)	15(5)	5(2) 4(2)
3	MRKAd5mCMVFLnet (E3+)	10.4.45	1	115	46	33	3(2)	3(2)	
		4010.00	2	132	42	32	4(2)	83(13)	5(1)
4	MRKAd5mCMVFLnef (E3+)	10 ⁴⁹ vp	ī	132	42	32	2(1)	29(2)	4(0)
			2	132	42	32	3(2)	14(2)	5(1)
5	MRKAd5mCMVtpanes(E3+)	10^7 VP	1	100	Ō	0	3(1)	13(4)	10(3)
	l				170	98	3(2)	145(29)	4(0)
6	MRKAd5mCMVtpanet(E3+)	10^9 VP	2 1	230 115	46	33	7(1)	151(14)	10(0)
				150	78	52	21(2)	· 18(6)	28(3)
7	Naive	none	none	152					

^{*}GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

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Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

Near or at the upper limit of the serial dilution; hence, could be greater than this value *No. of Spot-forming Cells per million splechoytes; mean values of triplicates are reported along with standard errors in parenthesis.

No. of spot-forming cells per million splecnoyles; mean values of triplicates are reported along with standard errors in parenthesis.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10	Macaques.
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viacaques.									Te7			T=16	
Vaccine (T=0,4 wks)	Monk #		Prebleec			T=4							B-16
		Mock	PolL	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKACETOMV-lApce(E3+)	99C100	1	0 2	0 2	10	38 98	31 249	0	52 109	146 305	22	49 68	715 250
10^11 vp	99C215 99D201	5	5	4	6	149	85	, i	40	35	ō	35	18
MRKAc6hCMV-IApal(E3+)	99D212	0	2	0 2	4	331 19	114 192	0	58 38	14 156	0 5	6 38	6 108
10% vp	99D180 99C2D1	8	5	21	6	62	62	ŏ	18	32	ĭ	14	65
MRKACENCMV-IApod(E3-) 1041 vp	99D239 99C186 99C084	5 4 1	2 12 8	2 6 9	20 5 8	82 120 84	172 421 464	1 2 0	68 271 14	114 489 236	9 16 1	21 875 24	40 530 264
MRKAGSHCMV-IApad(E3-) 10/9 vp	©7C ©16 ©11	10 2 6	10 0 6	8 1 12	12 5 10	724 474 98	745 468 110	4 0 5	322 232 60	376 212 80	4 0 8	188 101 25	176 121 34
Nave	0830	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined Reported are SFC per million PENICK; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

Vaccine/Monkey Tag	1=4	T = 7	T=12	T=16
MRKAd5hCMV-IApol(E3+), 10^11 vp				
99C100	61	1999	5928	4768_
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IApol(E3+), 10^9 vp				
99D212	10	40	49	68_
99D180	<10_	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IApol(E3-), 10^11 vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
990084	235	2637	2858	1626
MRKAd5hCMV-IApol(E3-), 10^9 vp	 			
CC7C	32	175	306	235
Φ16	20	140	273	419
<u>Φ11</u>	15	112	149	237

PCT/US01/28861 WO 02/022080

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Table 14.

Ma	caq	ues.

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Vaccine (T=0,4 wks)	Monk #	Pr	e	Ta	4	T=	:7	To	
Vaccine (120,4 WAS)	MOIN I	Mock	Nef	Mock	Nef	Mock	Nef	Mock	Ne
	CD2D	0	4	31	440	4	368	1	25
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC7B	اةا	Ď	2	521	0 1	178	1	152
10^11 vp	CC61	2	9	31	112	0	108	11	10
15 O O O O O O O O O O O O O O O O O O O	CC2K	9	9	6	52	0	35	0	15
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CD15	5	4	30	998	2	586	0	43
10^9 vp	CD16	6	1	6	1146	0	369	1	21
	000101	+	5	4	614	0	298	2	41
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	4	6	5	434	اةا	1100	2	93
10^11 vp	99D144 99C193	1	2	Ĭ	58	1	22	0	6
	99D224	+ -	11	14	231	1	125	0	7
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	990250	B	9	4	108	0	54	0	1
10^9 vp	99C120	1	6	20	299	0	92	0	7
Naîve	083Q	nd	nd	18	22	4	5	2	

EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nefb) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

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subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
	1 ((from mapping)		3-3-3-			,,,,,,
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	- 5	1055	1080	2210	2140
	_]			

EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef

Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer	AEX Titer	Amplification
	(10 ¹⁰ vp/ml culture)	(10 ⁴ vp/cell)	Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

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Roller Bottle Passaging - Passaging of the pol and nef constructs continued 5 through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements. 10

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

7. Ра Г	assage SIX \ Xviable (10	/ iral Produc	Cell Passage	AEX Tites	1103	Amplification	Triton Lysis Titer	
	Viabili Infection	ty (%) Harvest	Number	(Cell Associated)	10 ⁴ vp/cell	Ratio	10 ¹⁶ vp/ml culture	
	1 22 85%		62	0.8	0.7	25	1.6	
poor	1.22, 03 %							
1		0.99, 62%	}			Į į		
2		1.10, 72%	}			L		
pool	1.42, 89%		62	4.5	3.2	115	7.0	
		1.22, 70%	T			l I		
2		1.42, 74%						
	pool 1 2	Xviable (16 Viabli Infection pool 1.22, 85%	Xviable (10 ⁴ cells/ml), Viability (%) Infection Harvest	Xviable (10 ⁴ cells/ml), Viability (%) Number	Xviable (10° cells/ml), Viability (%) Number Cell Passage (Cell Associated) 10° vp/ml culture	Xviable (10° cells/ml), Viability (%) Number (Cell Associated) 10° vp/ml culture 10° vp/ml culture 10° vp/ml culture 10° vp/cell	Number Cell Associated Number 10 ⁴ vp/cell Ratio	

Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef 15 Triton Lysis Titer Amplification AEX Titer Xviable (10° cells/ml), Cell Passage (Cell Associated) Viability (%) 10th vp/ml culture 104 vp/cell Ratio 10 to vp/ml cultum Harvest Number Infection 2.1 29 0.8 1.0 66 1.33, 90% hCMV-FL-ocf [E3+] 0.96, 70% 1.18, 73% 6.5 168 4.7 4.2 56 0.90*, 90% bCMV-FL-pol [E3+] 1.18, 88% 1.04, 80%

MRKAdSnef and MRKAdSpol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6® cells in roller bottle cultures were infected at an MOI of 280 20 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 25

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

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Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10 ⁶ cells/ml), Viability (%)		Cell Passage	AEX Tites	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ³⁰ vp/ml culture	10 ⁴ vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nef	Pool	1.11,91%	1	60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23, 75%					
	2		1.34,74%	İ			ŀ	
mCMV-FL-nef	Pool	1.11, 91%	T	60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

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EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x10° cells/ml. Cells were grown until they reached a cell concentration of approximately 1x10° cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

1 201	20. Experimental Contracts	
Temperature	36.5 °C	
DO	30%	
	7.30	
PH	150 rpm	
Agitation	None	
Sparging		

Table 21: Virus source used for experiments.

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Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
112	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

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Table 22: Virus Concentration as measured by the AEX assay

	Batch ID	Cloned/Uncloned	v	irus Concentration @	48hpi (1x)	0 ¹³ vp/L)
Run	Batch	MRKAd5nef	Supernatant	Clarified Lysate	Total	Triton Lysate
	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
#1		Uncloned	0.38	1.67	2.05	2.46
	B20010115-2	Cloned	0.80	6.00	6.80	8.88
#2	B20010202-1		0.50	6.00	6.50	8.47
	B20010202-2	Cloned	0.50	0.00		

Table 23: Virus Titers as measured by the QPA assay

Patch ID	Cloned/Hncloned	Virus Concentration @ 48hpi (1x10 ¹¹ IU/L)							
Daten ID	MRKAd5nef	Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate			
P20010115 1	Unclosed		1.12	1.76	2.88	11.28			
			0.73	1.54	2.27	5.86			
				1.62	2.69	11.89			
			1.17	1.70	2.97	12.47			
	Batch ID B20010115-1 B20010115-2 B20010202-1 B20010202-2	B20010115-1 Uncloned B20010115-2 Uncloned B20010202-1 Cloned	MRKAdSnef Whole Broth B20010115-1 Uncloned 0.13 B20010115-2 Uncloned 0.14 B20010202-1 Cloned 0.14	MRKAd5nef Whole Broth Supernatant B20010115-1 Uncloned 0.13 1.12 B20010115-2 Uncloned 0.14 0.73 B20010202-1 Cloned 0.14 0.97	MRKAdSnef Whole Broth Supernatant Lysate Clarified Lysate B20010115-1 Uncloned 0.13 1.12 1.76 B20010115-2 Uncloned 0.14 0.73 1.54 B20010202-1 Cloned 0.14 0.97 1.62	MRKAd5nef Whole Broth Supernatant Lysate Clarified Lysate B20010115-1 Uncloned 0.13 1.12 1.76 2.88 B20010115-2 Uncloned 0.14 0.73 1.54 2.27 B20010202-1 Cloned 0.14 0.97 1.62 2.69			

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

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Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4⁺-biased or CD8⁺-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8⁺-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8⁺ T cells.

Į	Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkey	med Rhesus Monkeys wit	ys with MRKAd5gag	(Sgag															
Mum	Number of SFCAnifibar PBMCs						ŀ			1	-	15.17	-	T=24	\mid	T=28		1=30	
		Room	Monky	-	_			=			1	-1	İ	IL.	F	Ļ	۴	1	-
5		T-2R whee	=	Fed to	100	Hedium	1	Medism	H H Bad	Redicin	OND H	Ē	1	ξİ	Lifes	Ħ		1	
	I EU, 4, 6 WALE	OVA CALL	t	1		r	¥	r	7	7	22	-	±15~	_	2	-	200	-	0
[DNAS mas	MFIXAd5gag(E3+)	5	Š	 E	_	3 ;	_	-	_	8	-	76	_	35	_	705	_	755
	583	10^7 vp	ğ	0	<u>-</u>		9		? ;	> 1	3 4		2	· œ	98	2	686	•	395
_	(010)		AW30	S.	=		 8		5	,	 }		}	_	-	_		ļ	
_				1	1	1		ţ	1	ţ	270	•	San	L E	226	┝	626	19	1345
ľ	DNA6mas +	MRKAd5gag(E3+)	<u>8</u>	0	+	_	3	-			i		2		100	_	1915	_	660
_	_	10v7 vp	<u>8</u>	•	0	_	5 !	-	Š,	•			} =		2	=	23		241
			AWS	0	6	-	₽	•	Ξ,		0	 •	5 (-	3 2	_	15.40	- 5	7.30
			TARC	Ą	ž	0	_	0	28B	-	2		2			_		-	
_			AKAB	•	12	4	8	_	119	0	6	<u> </u>	425		316	-	677	0	<u>.</u>
_			}	,	!			_			_		1	1	1	+	+	1	
_				ķ	ŀ	-	8		ž	19	425	9	5	6	203		265	-	4
ľ	DNAS mast	MFRAd5gag(E3+)	AWG		,	- (3	, •		•	9	u	8	-	505	-	1384	•	B/6
_	CH1 1005/7 5 mas + 0.6 mM BAK	19v7 vp	ž Ž	_	>	•		- (3 5	. ,			200	-	208	_	636	-	828
_			9983	•	9	-	۱ م	n •	2	- ·			3		25	_	643	-	349
			MSBO	4	6	0	8	-	5	>	a c	٠.	3 8		750		22.R	-	183
_			0282	-	•	0	8	•	318	_	- }		3	-		_			
_						_					1		+	 	ļ	ļ.	-		٠
		Stone	SED 201	-	6	0	0	_	0	0	0	-	-	-	2	4	,	,	
Ĺ	none	Mond																	
	- At Hallen																		

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

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The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

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protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized

HIV-1 gag, pol, gagpol, nef in rhesus macaques

<u> </u>	ng, pol, gagpol, nef in rhesus maca	Monk #			T=6 wks		
irp#	T=0, 4 wks		Mock	Gag H	Poi - 1	Pol - 2	Nef
	MRKAd5 gag	CB9V	0	15		-	-
1	10/10 Vp	CD19	0 .	374	-	•	•
	10-10-46	109H	1	843	-	-	-
	MRKAd5 gag	99D130	1	948		- 1	-
2	10^8 vp	W277	16	324	-	l - I	-
	10.0 Ab	143H	4	595	•	-	•
	MRKAd5 pol	CC1X	4	 	46	256	-
3	10^10 vp	AW3W	3	١.	463	550	-
1	10-10 Ψρ	AV43	6		95	1333	-
	MRKAd5 pol	AW38	1	-	19	30	-
4	10^8 vp	CC8K	0	-	50	995	٠- ا
	1004	CC21	1	-	33	436	-
5	MRKAd5 nef	076Q	9		1 -	-	1204
٥	10^10 vp	091Q	4		-	-	85
	10.10.4	083Q	0	1 -	-	-	176
6	MRKAd5 nef	000029	1	1	-	-	114
۱۳	10/8 VP	98D022	6		-	1 .	170 198
ļ		98D160	3	1	1	1	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120
_ ′]	10^10 vp each	05H	3	135	21	9	638
ļ	10 10 14	00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nel	99D215		171	18	193	240
•	10^8 vp each	81H	5	73	6	14	
	72.2	12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef	99D211		83	56	838	725
3	10^10 vp each	22H	4	385	119	1194	191 853
	,5 75	61H	4	343	11	765	85%
10	MRKAd5gagpol +MRKAd5 net	34H	3	78	19	5	75
10	10/8 vp each	48H	1	65	105		43
	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	70H	5	158	15	220	19

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10^6 PBMC.

WHAT IS CLAIMED IS

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A recombinant adenoviral vaccine vector at least partially deleted in
 E1 and devoid of E1 activity, comprising:

- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
- b) a gene encoding an HIV protein or immunologically relevant modification thereof.
- A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- 3. A vector in accordance with claim 1 further comprising nucleotides
 15 corresponding to between from about base pair 3511 to about 3524 to about base pair
 5798 of a wildtype adenovirus genome.
 - 4. A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs451-3510.
 - 6. A vector in accordance with claim 1 which is at least partially deleted in E3.
 - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

- 9. A vector in accordance with claim 1 wherein the vector comprises a5 gene expression cassette comprising:
 - a) a nucleic acid encoding a protein;
 - b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
 - (c) a transcription termination sequence.
- 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
 - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
- 12. An adenoviral vector in accordance with claim 9 wherein the gene
 expression cassette is in an E1 antiparallel orientation.
 - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
 - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
 - 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.

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16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.

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- 20. An HTV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises aphysiologically acceptable carrier.
 - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 23. A method according to claim 22 wherein the cell is a PER.C6® cell.
 - 24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
 - 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

- 27. A method according to claim 24 wherein the adenovirus vaccine is
 5 preceded by an adenovirus vaccine of a different serotype.
 - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
- 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
 - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
 - a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising

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- i) SEQ ID NO: 29;
- ii) a heterologous promoter operatively linked to i); and
- iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

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- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
 - 37. A cell comprising the adenoviral vector of claim 30.
 - 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
 - 39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.
 - 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
- 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

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- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
 - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
 - 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene
 20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
 - 50. A recombinant adenoviral vaccine vector at least partially deleted in B1 and devoid of B1 activity, comprising:

a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

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- b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of
 SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
 - ii) a heterologous promoter operatively linked to i); and

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- iii) a transcription termination sequence.
- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 20 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
 - 56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

- 58. An HIV vaccine composition comprising purified adenovirusparticles of claim 57.
 - 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
 - 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

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- 61. A method according to claim 60 wherein the cell is a PER.C6® cell.
- 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
 - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

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- 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
- 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
 - a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - i) a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
 - 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

- 72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

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- 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
 - 75. A cell comprising the adenoviral vector of claim 68.
- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.
 - 78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
 - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 80. A method according to claim 79 wherein the cell is a PER.C6® cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

- 82. A method according to claim 81 which further comprises

 5 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

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- 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
 - a) gag, pol, and nef, expressed independently from three individual vectors;

b) gag, pol, and nef, expressed independently from one vector with
the encoding nucleic acid sequences operatively linked to distinct
promoters and transcription termination sequences;

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 gag, pol, and nef, expressed via two vectors, one expressing a polnef fusion, and another expressing gag;

- d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol;

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- f) gag, pol, and nef, expressed via one vector expressing a gag-polnef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

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- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

- k) nef and gag, expressed independently from two individual vectors;
- nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion;
 and

- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.
- 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.

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89. A multivalent adenovirus vaccine composition in accordance with

10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences

operatively linked to a single promoter; and the encoding nucleic acid sequences

operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

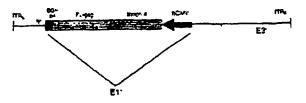


Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgttgctgttggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caagaagaagtacaagctaaagcacattgigtgggcctccagggagctggagaggtttgctgtgaaccctggc agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag gaggecciggagaagattgaggaggagcagaacaagtccaagaagaaggeccagcaggctgctgctggc acaggcaactccagccaggigtcccagaactaccccattgigcagaacctccagggccagatggtgcaccag gccatctcccccggaccctgaatgcctgggtgaaggtggtggaggagaaggccttctcccctgaggtgatccc catgitctctgccctgtctgagggtgccacccccaggacctgaacaccatgctgaacacagtgggggggccatc aggetgecatgeagatgetgaaggagaceatcaatgaggaggetgetgagtgggacaggetgeateetgtge acgetggccccattgcccccggccagatgagggagcccaggggctctgacattgctggcaccacctccaccct ccaggagcagattggctggatgaccaaccaccccccatccctgtgggggaaatctacaagaggtggatcat ccigggccigaacaagatig:gaggatgtactcccccacctccatcciggacatcaggcagggccccaaggag cccttcagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagaggggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga agggctgctggaagtgtggcaaggagggccaccagatgaaggactgcaatgagaggcaaggccaacttcctg ggcaaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagccct agetglaccccctggcctccctgaggtccctgtttggcaacgacccctcctcccagtaaaataaagcccgggca gat (SEQ ID NO: 29)

Figure 2

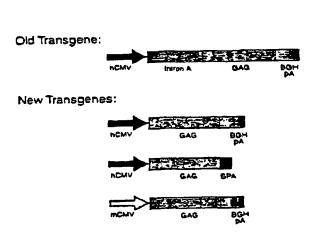


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

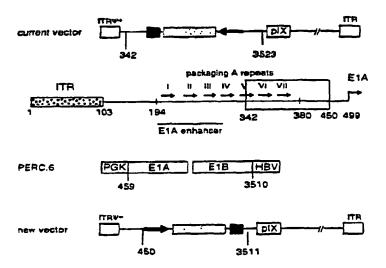


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

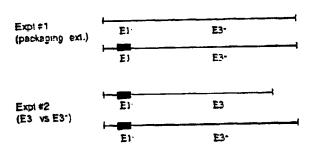


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.



Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

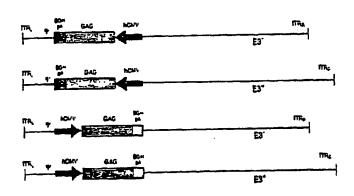


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

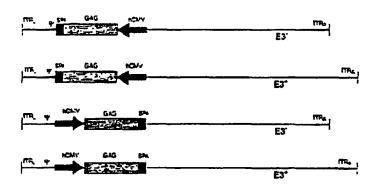


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

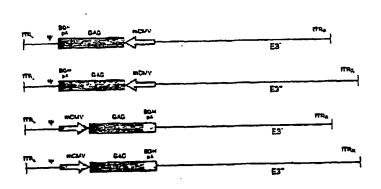


Figure 7C; mCMV-FLgag-bGHpA adenovectors constructed within the *MRK* backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

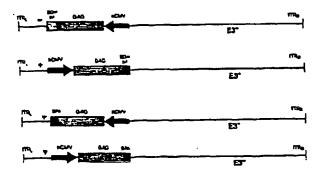


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

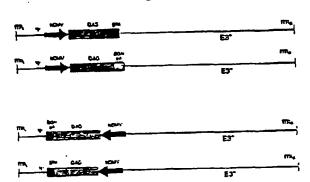


Figure 8B: Effect of polyadenylation signal

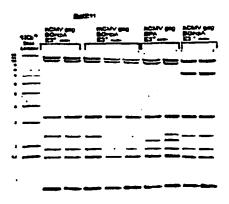


Figure 9: Viral DNA from the four Adgag candidates at P5, following EstE11 digestion.

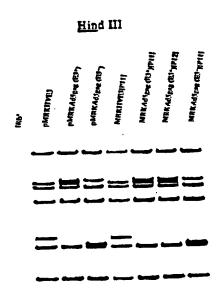


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

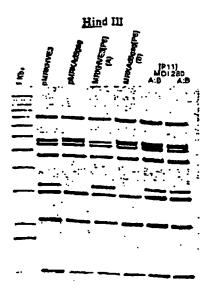


Figure 11: Viral DNA analysis (*Hin*dIII digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

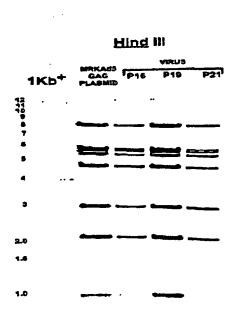
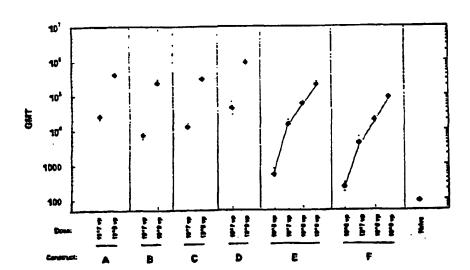


Figure 12: Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb'c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3 bCMV-FLgag-bGHpA; (C) MRKAd5 E3 bCMV-FLgag-SPA; (D) MRKAd5 E3 mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-lgag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-lgag. Reponed are the geometric mean titers (GMT) for each cohort.



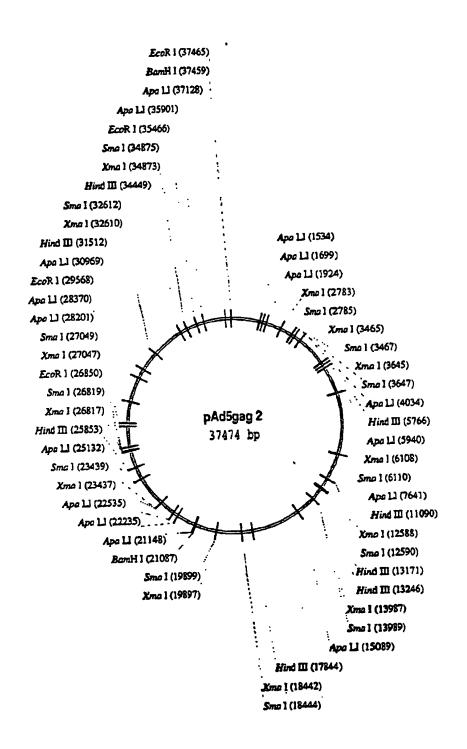


Figure 14

						4170		Andreador & Carle		CHARCEARCIES
-	· TTCTTAATTA		-				מעייט ויייני			a Cocamon
	ACATTAAT	TUTACTACT	ATTATATES	ATANANCUTA	ALTIN YASTTA	TACTANTACT	טער בכע ער כווב			באכוני וויאים
101	CARCOCACTOAC	CTAGTAGTE	CATCOTANTIC	TriATriTriCA	ACTUTATION	AACACATGTA	ACCIONATION	CTCCCANANG	TCACOTIFIED	מנונטת שנוני.
1	CCCCCACTG	CATCATCACA	CCCCCTTCAC	ACTACAACGT	TUNCACCCCC	TYCTGTGTAFAT	TOTOCHOOCITA	CACCOTTITIC	ACTOCANANA	CCVCVCCCO:
201	CATTOTACACA	COAAGTOACA	ATTTRICKENE	CEPTTAGG	GRATGETA	GTAMATTICG	CATCTAACCCA	GTANGATTE	OCCATITITICS	CTCCANANT
1	CCACATGROT	CCTTCACTOT	TAMAKECIN	CCAAAATCCG	CCTACAACAT	CATTITADACC	COCATTORICT	CATTCTAAAC	COSTANAGO	CCCTTFINA
101	CAATAACACT	AACTICANATC	TCANTANTIT	Trenche	ATATATA	ATATITGE	AGRICCERIOG	GGACTITICAC	corrracera	GASACTORA C
))	CHTATTETEC	TICACTITAG	_	ACAC:AATGAG	ナハ ザン がたに入す	TATAMACARA	TCCCOGCGCC	CCTGAMCTO	CCANATGCAC	CTCTGMGCP.
401	CAGGIGITH	TETCAGGIGT	THYCOCHT	CCURRETEAM	THEORYCETT	TATTATTA	פטינוסכננפנים	ATCCATTGCA	TACOFFICTAT	CCATATCAT
!	GTCCACAAAA	AGAGTECACA	_	GOCCCAGTIT	CANCICICANA	ATANTANTA	حصودووووو	TAGGTAACCT	ATGCAACATA	CCTATAINTA1
501	ATATGTACAT	TTATATTOGC	TCATCTCCAA	CATTACCGCC	ATCTICACAT	TGATTATTGA	CTACTTATTA	ATAGTAATCA	ATTACGGGGT	CATTAGETICA
,	TATACATOTA	AATATAACCO	_	CTMATGREGG	TACAACTGTA	ACTAATAACT	GATCAATAAT	TATCATTAGE	TAATGCCCCA	Grantchagt
601	TAGCCCATAT	ATGGAGTICC	GCOTTACATA	ACTITACIONTA	ANTERCECOC	CIRRICIANACC	שכככעשכניעכ	CCCCGCCCAT	TCACCTCAAT	AATGACGTAT
	ATCODOTATA	-		TCANTOCCAT	TACCORDING	GACCGACTES	coochocro	GROCCOOTA	ACTOCAGITIA	TTACTOCATA
701	GITCCCATAG	TAACGCCAAT	AGGGACTITIC	CAITRIACRIC	ANTORGINAA	GTATTTACCE	TANACTYSCCC	ACTTOOCAGE	ACATCAAGTO	TATCATATY
•	CANGGOTATC	_	_	OFINCTGUAG	TTACCCARRY	CATAMATIC	ATTTISACINGO	TGMCCGTCA	TOTAGTICAC	ATAGTATACC
108	CAAGITACKICC	_	_	CTUULATICACC	CCCCTTGCCAT	TATCHCCAGT	ACATGACCTT	ATCCCACTIT	ccractrosc	AGTACATOTA
!	GTTCATGCGG	_		CAIPTACCIO	GCFGACCETA	ATACCCCTICA	TGTACTGGAA	TACCCTGMA	GGATGAACCG	TCATGATAGAT
106	COTATTACTC	_	CCATCOTCAT	CCCCTTTTCC	CASTACATCA	ATGGGGGG	ATARXYRITT	GACTICACOO	DATTTCCAAD	Tenceveer
•	GCATAATCAG		_		GPCATKITAGE	TACRETATA	TATCCCCANA	CTCAGTOCCC	CTANAGGTTC	AGAGGTTCACA
1001	ATTRACTOR		_		COGACTITICS	AAAATISTOCIT	AACAACTCCO	CCCCAPTGAC	GCAANTGGGC	CCTAN XCCTI:
1004	TARCHECAGE		_		CCCTGAAAGG	TITTACAGCA	THOTHGAGGC	COCCHANCTO	COTITACCCO	CCATCCGCAC
1101	TACTORDAY	_				GCCTGTAGAG	GCCATCCACG	CTOTITIOAC	CTCCATAGAA	CACACCCITISA
	ATOCCACCET		-			COGNICITY	COSTARSTOC	GACANAACTO	GAGGTATETT	כופופטכריי
							Rgfi			
1201			GOOMFOOTH	CATTOOMCG	COGATTCCCC	CHISCEARCAG	TCAGATICTAC	CATGGGTGCT	AGGGCTTCTG	TGCTGTC.TGG
	COCTACCTCG	_	_		GCCTAACAGG	CACCATTACTIC	ACTICTAGATO	GTACCCACGA	TCCCGAAGAC	ACCACACACACC
ן סני ד	•	-		CCTUMOCACCT	CHILLETANGA	AGANGTACAA	CCTANANCEAL	ATTOTOTOGG	CCTCCARRRA	CKTRACIANAG
*	_				CCACCGTTCT	TUTTONIOT	CGATTTCGTC	TANCHERECE	COACCICCT	CCACCACAC
1401		_	_	_	CCAGCCAGAT	CCTGGGCCAG	CTCCAGCCCT	CCCTOCALAC	AGGCTCTGAG	CACCTGAGGT
7067	PRACTIACIACIA			-		CATACCCCACTC	GAGGTCCCCA	COGACOTITO	TCCGAGACTC	CICCACTICIA
1001	•	_		CHCTYCACCA	GANGATTGAT	CITCAMAGACA			ATTONOGAGO	ACCAGANI 'AA
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1601	•		. ARKETERE	TOTACACACAC	AACTICACKT	ACATHOTECCA			ACCTCCAGO	CCAGATGGTG
· >				_	ACCONTRACTO TIVIAMETER	TCACACACCT	CFFCATCOCG	TAACACGTCT	TOGAGGICCC	GOTCTACCA!

Figure ISA

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CATOTTCTCT GTACAAGAGA ACCATCAATG TGGTAGTTAC	CTOCCACCAC GACCOTGGTO GAACAAGATT CTTGTTCTAA	CTGAGGGCTG GACTCCCGAC TGGGCCCTGC ACCCGGGACG	GACCAACTEC CTGGTTGAGG AACTGTAGGG TTGACATECE	TCTGGCCTC AGACCGGGG CACCCCAGC GTGGGGTCG		GNCANTAGCA CTGTTATCGT		00 to 10 to
AGATGATCCC TCCACTAGGG GCTGAAGGAG CGACTTCCTC	TCTGACATTG AGACTGTAAC TCCTAGACCT AGGACCCGGA	CTACAAGACC GATGTTCTGG CTGAAGGCCC GACTTCCGGG	TOTECCAGGT ACAGGGTCCA CATTGCCAAG GTAACGGTTC	CTCCCATAT GACCCATATA AGGACAC TCCTCTTCTG	CATTITATIT CATTITATIT CCACTOTCCT GGTCACAGGA	CCTANCCCTT	AGNATATATA TCTTATATAT	TATAACTOT TATAACTOT TTGACCTACG ANCTGGATGC
THETCECETG AAGAGGGGAC CEATCA AGAT GGTACGTCTA	CCMGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	TGGACAGGTT ACCTGTCCAA CAAGACCATC GTTCTGGTAG	GCTGAGGCCA CGACTCCGGT AGGTGGGCCA TCCACCCGGGT	GCGGTTGAAG AGGTTTGAAG	CCTCCTCCCA GGTVCCACTC	COTTCCCCT	AGGGTGGGAA	A ANCACTCOAG A CTCTACTACC F GAGATGATTO
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GCCTVAXGTGA CCXGAGCCACT TYXTTGAACAC ACCAACTTTTG	TOTACTOCANT ACCORDINATOR CCCCCCATCT CARROCATOR CARROCATOR	ACCCCCCCTTCCAACCCCCCCCCCCCCCCCCCCCCCCC	GCCCCGGGAC GCAAGACAGT GCAAGACAGT	CCACCARATO GGTGGTCTAC GAGCCCACAG CTCGGGTGTC	CCTCCTOAG GCACCCCCC CCCTCCCCCC	ACCICATOR CHARTCHAS CONTRIBUTE CECTATAGE TECHNOLOGIA GATAMGACEE CECACECECA CECGTECTST PAUL	Asci is cecentarite ic ecoecaticae	CCATTANTCAC GCTACTTCTTG CATTGATGTT GTAACTACCA
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TCTCCCCCG AGAGGGGGGC CCCCCARGAC GGGGGTCCTG	ACCCACCTAGA TECCACCTAGA TECCACCTAGATAGATAGATAGATAGATAGATAGATAGATAG	CTCCATCCTG GAGGTAGGAC AAGAACTCGA			CAAGAGCTG GTTCCTCGAC GCCAGCCATC CGGTCGGTAG	TTOTCTUAGT AACAGACTCA	GOCTCTATOO CCGAGATACC	HTTTGCAGCA SAMAGGTCGT CAGAATGTGA OTCTTAGACT
CACCAGGCCA GTGGTCCGGT ACGTGCCAC		ACTCCCCCAC TOAGGGGGTG CCAGGAGGTG	GAGGAGATEA CICCTCTACT TGATGCAGAG	GANGGOCTGC CTTCCCGACO AGGCCTGGCA TCCGGACCOT	ACCCATTOA TCOCOTAACT CCTTCTAGTT GOAAGATCAA	TTOCATCOCA	OGATOCOGTG CCTACGCCAC	TRIGIANTERO ANCATAGAC CROGGIGGGT GCCCCACGCA
1701	1901	2101	2301	2501	2801	2901	3001	3201

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3301 3401 3501	TTOOMCACTO ANCETETOM CTTCCOTTC GANGGECAO TETGCOCAO	ARCETECTS TECTOMOGIC ATECOCECCS FAGGENGICS	CCCCICTITEN GCGCCIAAGT GATCACAAGT CTACIGTICA	CAYTATURE CERTATURES CHRISTELL (REPRESENSES RINGERATET TERRESCAFA ACFRICKRATA AAACGSTITT THYTHXCOTF CCCAATHRES	CCACTORICS OFFICE ACTORICS TITUS CACTOR MACCORTIT	CYPGATTGEG GTTCTWENG THEFATTCTT ACCTWENA	ACTESACTTES TEACTESAME TEACECETES ACTEGRICCET	CHITICHOAD GAMMONCTC ACTITANTOTC TGANTTACAG OCAGACTOTG	CCCRCTICCA GRICOAACOT GITTCTCAGC CAAAGAGTCG	AACMITGEAG TTTTGACGTE ACTVITTGA TCGACAAN T
3601	AGACGCGGTC GTGTCTTGCT CACAGAACGA	GTCCAAAGAC GTCTTTATTT CAGAAATAAA	GGGACTTGGG AGGGGTTTTG TCCCCAAAAC	AAGGAAGGAAA CTACKCACACA	GENTALING ARGCCCHRA TCCRAGCCCT	AAATTTRITA CCAGGATICT GGTCYCCAGA		OCTCTGAGAC OCCTCCT0TG CCCAGGACAC	AAACCTAAAC TATTTTTTCC ATAAAAAGG	CTACITYCE (*) ACCACCACCA TCCTGCACCA
3701	AAAGGTGACT TFFCCACTGA GATCCAGTCG CTAGGTCAGC	CTOGATOTIC GACCTACAAG TAGCAGGAGC	AGATACATGG TCTATGTACC GCTOCACGTG CGACCTTCAC	CCATAMOTIC CCTATTCOM GTGCCTAAAA CAIGGATTTT	CHCHCHCASS CACINIACCY C APPRICATION TACASANGE	THEMOGRAMS ACTICEATEG GTACCAAGCT CATCGATCGA	NCCACTIFICAD ADCTTCATGC TOCHGACGTC TOGAAGTACG GATTISCEMGG GOCAGGGCCT CTAACGGTC CCGTCCGGGA	AGCTTCATGC TCGAAGTACG GGCAGGCCCT CCGTCCGGGA	TCCCCCTCC ACCCCCACC TCCTCTAAOT ACCACATTCA	TOTTOTAGAT ACAACATICTA GTTTAGAAN: CAAATGTTTG
3901	COSTTANGET OCCANTICGA TOTTOTOCAG	CCCTACCCAC AACCACCAC	CATACGECCC OTATGCACCCC ACAGTGTATC	CATATRACAT CTATACHCTA CCCTCCACTT	GCATCTTARA CGTATANCCT GREGNATTAG	CHOTATTTTT GACATAAAAA TCATATAGCT	ACCIATORETA TCCAACCGAT TAGAAGGAAA	TOTTCCCAGC ACAAGGGTCG TGCGTGGTAG	CATATCCCTC GTATAGGGAG AACTTGGAGA	CORRESPONDA GCCCCTAMAT CRCCTTGTVI
4101	ACCTCCANGA TOTACCAGGA ACAGGGTCT		ATTCFICENT TANGCAGGTA ATNXXCATT FATCCGGTAA	ANTENTOGO TTTACAANGC AAATGTTTG	ATTACCOCUTO TACCCOCUTO GCCCCCCCCCC CGCCCCCCCCCCCCCCCCCCCCCCC		CTTGGCGAAG GACCCGCTTC TGCGGTATAA ACGCCATATT	ATATTICEGG TATANAGACC TOGITCCATC	CATCACTAAC CTACTCATTO COCCCCACOO OCCCCCACCC	GEOTAGTTA GEOTAGTTA GEOTAGTTA GECTAGTAN:
4301	CCTCACAGAT GGAGTGTCTA GTGGGAAGAA	PTGEATTICC AACGTAAAGG AACAGGTECC		GTTCAGATOG CAAGTCTACC CGACTTACCG	CCCCTAGTAC CCCCTAGTAC CAUCYGGTTAG			CTTTTGCCAA	ACTION AGENCY AGENCY ACTION ACTION AGENCY AG	CCCTCTAGTC Pstl
4501	Pass CAGCTGCCGT GTCGACGGCA		TEGICCANOG ACICCICONC OCTIONINAL GILOGECALE. CATECCTGAG CAGGAGGEC ACTICGITIAA GCATGTCCCT GINGOGACTE GICCECCCG IGAAGAATT CITACAGAAA	ACTICUITIVA ACTICUITIVA TGAAGCAATT	GCHIMNIAC CHUMACUAL ACHEGITIM GCATGICCT TGMGCANT CATACAGAA	CHARLESTAND TETTECCTEA CTEMECSTANC ANAMOGANCT CTEMECSTANC ANAMOGANCT CTEMECSTANC ANAMOGANCT CTEMECSTANCE CTEM		CCAAATCCOC	CAGAAGGCGC	TCGCCGCCA ACCCGCGGGT
4701	OCCUPATIONS CECTANCOTO CCACAOCTOS GGTUTCOAGO CCAGACOGOS GGTCTGCCCO	TTCTTOCANG ANGANCOTTC OTCACCTOCT CAOTGANGA CAOCCTOCT	CTECETTICA CTACGACATC CTACGACATC GATGCCTAG TCTTTCCACG AGANAGGTGT	TTTTCAACTO AAAAGTTGCC TCGATCCAAC AOCTAATTCC GAXYTVAACT	TTTGAGACTOTATIC ATATCTCCTC TATATATATATA CCTCTTTTCG GCGACTOTATICG GCGACTATATATATATATATATATATATATATATATATAT	HTCCCCTTAG NTHICTCTAG GALVACICIG GALVACICIGG GALVACICIGGG CATACTICTIGGG CATACTICTIGGG CATACTICTIGGG CATACTICTIGGG	GCANY CTTTT COTACOAAAA TTGAACCACC AACCCCCCC TCACATCACA AACCCCCCC AACCCCCCCC	GACCOTTGA CTCCCAAACT TTTCCCTGTA AMCCGACAT GCCCACGCGA	CCANGCAGTT GGTTCGTCAA CCCCAGCATCA GCCGTCATCA CCCGGCCTCCA	CCAOCCOTC GATCCCCAT CGATCCTCTT GCCACGATA:A CCCTCGATA:A

Figure 150

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NAM GRECAGGESE GRE GERAFRAMA CCG COCTUTTAN CAM AMAGENETITAN CAT THIRGTEEN: (GTT THIRGTEEN:	GOTTH TOTAL ACTION TOTATION TATATION TO CONTINUE CONTINUE TOTAL AND TOTAL AN	D AMMINISTA C TTCCTCCAT D TANTITITY C ACTANCAM	A CHOROCOTIC A TTCACCTOTIC T ANTHOGACY!	A COCOCARCOT			F ATTCCANING TO TARGOTTCTA TO TARGOTTCTA TO ATTCCCTCC TO GARACTTACK:
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GATARCHERA GTARACTOOF THARAGCOTA ACTECCOCAT CTCTGGCCOT GAGACCGGCA	GOTTHICGAAA AGGCTGTCCG CCACTGCTTT TCCGACAGGC	CTCGCGTCCA OAGCGCAGGT CTCTTCGGCA GAGAAGCCGT		AGAMAGACA ATCTTTTAT TOTCAACTT GOTGOCAAAC TCTTTTCTCT TAGAMAACA ACAGTTCGAA CCACCGTTTG Pvd			A COTAGNOSOS CTCTCTONGT DOSOTCOGOS CCONGOTTOS CTCCAGCCCT GOCTCCAACO 7 ACOTTODAGE TEXEOTETOT TOCAACTTOS ACCOCAGACA
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CONTROL OF CONTROL OF	GACCACACTC CACATACGAG	GAACCACTCT CCTTGTGAGA GTGTGAAGAC	GCCACCCCC GCTAAGATTG CCATTCTAAC	ATCTTTTIVIT TAGAMARCA	OCTCCTTORC CGAGGAACCG ACCGGGTTG TGACGCCAAC	GATGETANGAG CTCCCATCCC GCAAGTCTAG CTTTCAGATC	CARTACCICAN GTACCICCTT AITTCOTICCI TCAAICACCIC ATATACCAACC
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CCATCCOANC 1 TCCACCCOANC 1 TCCACCCCOA C CCACCCCCA C CCCATCCCO C GCCATCCCO C	TCCCCCATGC AGGGGGTACG	AGAGGCCTGT TCTCCGGACA AGTGGGAGGG	TCACCCTCCC GTAGGTGTAG CATCCACATC GCCAGCTGTT COGTCGACAA	CCGCGGTGAF	CACCIACTES GEOTFSAAC CATTCOGAA	GTAGGCGCTC CATCCGCGAG AAAGACCCCG	OCCUPACIO CCCANCTCAC ATCTAGGOTA FACATCCCAT CTGCTCTGCT GACGAGACGA
4901 c 5001 1	5201	5301	5501	5701	5801	6001	6201

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6501	CECOTCACGEA	COANGOAGGC	CATCCTCAGE	COCACCITICAT	AND CHARTER OF CHARTER ACTIVATION OF CHARTERS ACTIVATIONS CHARTERS	CHEST ACTOR	THICACHTCTA CADGCCACTA ACHTGCAGAT CCCCCCGTCAT		GTCCAGAGIT	TECTTGATEA AGGAACTACT
6601	TOTCATACTT	ATCCTUTCCC		ACAGCTICKESS TISTICEAGEGE	CAACHEECTA	אארדרידראה דידואיזאניני	CCAGAMOOT	GTACTCTF00 CATGAGAACC	ATCCCAAACC TAGCCTTTGG	DEMERCIANE.
6701	CGAACOGTAA	GNGCCTAGCA CTCGGATCGT	TGTAGAACTG ACATCTTGAC	GTTGACGACC CAACTGCCGG	TOTATECTOR ACCOUNT	ALKTATCT CTT TXXITAGGGAA	THETACKAGT	AGCGCCTATO TCGCGCATAC	CCTOCGCGCC	CTTCCOIAG GAAGGCCIV'I
6801	CTCCACACCC	TGACCCCAAA ACTCCCCTTT	GOTOTCCCTO	ACCATCACTT TEGTACHGAA	TRINGITAL TVI ACTRICATIVIAL	CATAMOTTE	TCAGTGTCGT AGYCACAGCA	CCCATCCGCC	CTGCTCCCAG	ACCAAAAAGT TCGTTTTTCA
6901	CCOTOCOCTT	TTTOGAACGC	OCATTTGGCA CCTAAACCGT	CCCGCTTCCA	CACATYCHTE:	AAGAGTATCT	THY CCCCCCCC	ACCCATABAG TCCGTATTTC	THECOTOTION	TGCGGANGG: ACGCCTTCCX
7001	TCCCGGCACC AGGCCCGTGG	PEGGAACOOF ACCTTOCCA	TOTTANTTAC ACANTTANTO	CHOCHCOCOCO	AGCACGATCT TCGTGCTAGA	CCTCAAACCC OCACITTECGO	CANCTACANC	TOCCCCACAA	TOTANGTIC ACATTICANG	CANGAAGCG" OTTCTIV: GCV:
7101	GGGATGCCCT	TONTOGANGO	CAATTITITA	AGTTCCTCGT TCAAGGAGGA	AGGTGAGGTC TCCAGTCGAG	TTCARXXXX AND TTCARXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	CYCAGCCCGT	CCACACATTIC CCACACATTIC	GOCCCAGTCT	GCANGATICAG COTICTACTIC
7201	CCANCETTED	CHECHANICAD CTGETTACTE	CTCCACAGGT	CACTAGGCCAT	TAGCATTICE ATCGTAAACG	ANGTOGRACIC TECALECAGES	GANAGOTICCT	ANCTOCCGA TTTCACCCCT	CCTATOOCCA	TTTTTTCTCC AAAAAGACC
7301	CCACTACGTC	TACANGOTAA	OCCIONATION COCCOCAGANC	Treceages Angotese	TCCCATCCAA AGGGTAGGTT	CELANACECES	TAGGIC: TCGC ATCCAGAGCG	OCCCCTCAGT	CTAGAGGCTC	ATCTCCCCCO TAGAGGCGGC
7401	AACTICATOA TTOAAOTACT	CCACCATGAA F GOTCOTACTT Prod	GCGCACGMIC CCGGTGCTCG	TCCTTCCCAA ACGAAGGGTT	AGGECCECAT TECEGGGGGTA	CCAAGTATAG GGFTCATATC	GICTCTACAT	CCTARGOTGAC	AAAGAGACGC YTYCTCTGCG	TCGGTGCGATI AGCCACGCT
7501	CTACGCTCGG	GATCOGGAGO CTAGCCCTTC	ACTOGATET TTGACCTAGA	CCCGCCACCA	ATTOCANTAR TANCCTCCTC	TYSCTATTGA ACCSATAACT	TETEGRADA GTADAAGTCC ACACCACTIT CATCTICAGG		CHOCOCHOCOC	CCGAACACTC
7601	CACCACCCGAA	F THOTAMANC A ARCATITITIO	OTCCCCACTA CACGCCTCAT	CTCGCAGCCG GACCGTCGCC	TCCACTANCT ACOTTACCCUA	GTACATCCTG CATGTAGGAC	CALGAGGTTU	ACCTERCRAC COCOCACANO TOCACTECTS COCOCACTIC RMI	CCCCCACANO ACCCCTCTTC	GAA GCAGAGT CTTCGTCTCA
7701	CCCTTAMCT	A OCCUPAÇÃO	TCCCCCAAA	CCGACCACCA	CTTCTACTTC	CCCACCAACA	CCTTCACCGT CCTACTURES	CTURICTUCTO GANGGARTE GACCGACGAG CTCCCCTCAA	CTCCCCTCAA	ACCCACCTN !
7801	CCTOCTOCTO			AGATOTCCGC TCTACAGGCG	GENERAL CONTROLLAR	CTATACCTTATA	TEACAACATC ACTGTTGTAG	GCGCAGATGO CGCGTCTACC	GAGCTOTCCA	TOGICTOGAG ACCAGACCTC
1901	CYCCCGCGCC	CAGTECAGTE	OCCOPACETE COCCUTCGAG	Psil CTCCACCITT GACCTCCAAA	ACCTCGCATA TGGACCTTAT	GACCAGTCAG CTCCCAGTC Kph	GACCASTCAG GOCOCCAFGCT CTTCCCAGTC CCGCGCCCA KRAI	AGATCCAGGT TCTAGGTCCA		Trecompatic AAGOTECEEG
8001	TOCTTOCTOG		COCCACCAT COCTTCCANG	AGGCCCCANTC TCCGGCGTAG	הבניזכניותה הממזוכניותה		DACTANYANA CLANICANCE GOCOSTOGO CTONTONIA CANCOLOGO CTOCOCOCOCOCO	COCCACCC	COCOOCIOSTO TECTTRICATA	TCCTTCCATY:

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	OCCUPANCE CCCGGGGTTCC ACCOCCCCC TCCACCCCCCT ACCCCCCCCCC ACCCCCCCCC	A AGGCHTCTAA T TCCGCAGATT T GTAATTAANG A CATTAATTTC G GCTTCGTTTT C CGAAGCAAAA T CTATCATTTC A TAATAATTCC A TAATAATTCC
MOCOGNAC GCGTACCANT: TTCGCCACTG CGCCTATTTTTTTTTTTTTTTTTTTTTTT	GARTECTICA TATACCCCAAA CTAAGCAACT ATAGCGGGTT CCTCCTCCAG AAGACGGATG GGAGGAGGTC TTCTGCTTCTT CCGGAGGAGGA AGAAGAAGAA CCGCGGCGC GGCCCATAGT GGCGCGCTAG GGCGCGCTAG GGCGCGCTAG GGCGCGCTAG GGCGCGCTAG GGCGCGCTAG CCGCAGGGAT CCGAAGGGAT	LES SERVED EN
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ATACCATACTAN TACATAGATT MODACCTECH GCTTGANCCT CGANCTTGGA GATCTGGA GATCTCGGC CTAGAGCCCG TACGAGGAAGG ACCCTCTTCC CCACGTGCCG GGTCCTTCC CCACGTGCCG GGTCCTTCC	TCCCACCHC ACCOTTACA TCCCATACA TCCCATACA CTCCCATAAC GAAGGTATTC GAAGGTATTC CTACTCTCC CTAGTAGACG GTTCCCCCCCCCC	CATCUACCO GTAGTTET GTOTTET CAACAAGA ACTIACOCO CTCCTCCT GAGGAAGA CCCCCTCAT
8101 8301 8401 8501 8601	8701 8801 8901 9001	9201 9301 9401 9501

9701	ACAAACCCCC	OGTATOCOCC	OGTATOCOCC CONSTIGATO	GTGTAATTC	AGTHERICEAT TO BETTER	AACTICACTAG	TTAACGGTCT (CHOCHAGAGE	ACCACATOR:
	473976 AN	2								
9801	TGAGACGCGA	OTANGCCCTC G	OTANGECETE GAGTEAAATA	CKITAGICATIT	CALAACTECTIC	ALTENDITACIT GGTATCICCAC	GGTATCCCAC	CANANAGIGG	OCCONCCCCT	COCCUTACA:
	ACTICTISCOCT	CAFTCGGGAG	CAFTCOCOAG CTCAGTITAT	CCATCANA	COLLECAGE	TEATH CENTAGESTE FORM	CCATAGOOTO /	GITITICACO CCOCCOCCOA	ככפכנפכנמץ	ככפכניאונדי
000	COCCACACA	ACCONTOCCO	GOCTCCGG	CATCACANCT	TELANEATAN	CARCOATCATA TCCCTFMCATO		TACCTOGACA	TCCAGGTGAT	מכבספרהיובם
	CCCOOTCOCA	TCCCACCOGC	CCCCAGGCCC	CCCCTCTAGA	AGGITGIATE	CCCCTACTAT AMACATCTAC		ATGGACCTGT	AGOTECACTA	حوودودودود
10001	OTCCTCCAGG	CECTECODAM	GTCGCGGACG	CCASTTECTAGA	لديلديندي	CYTICAAAAAG	TGCTCCATCG	TCGGGACGCT	CHOOCEGOTC	
,	CACCACCTCC	COCCOCCTIT	CAGCGCCTYGC	COCCAMOGRACT	ACAACGCCTC	occammenc	ACCIACITACIC	ACCCCTGCGA	GACCOGCCAG	זככפנפכפני:
		Most						A a property C. A. A. C.	CONTANTANCE	CTSTACTERITY:
10101	TRACCACTO	CONCATCTOO	CACUTTICE	MGAGCCTISTA TCTCGGACAT	TCGCCCGTGA	CHICCHING	GACCACCTAT	TTAAGCGTTC	CCATAGTACC	
10201	CONTROPICO	CCCOTATECO	OCCOPECTACE	OTGATCCATG	CONTINUCCO	CCGCCTGTCG	ANTCCARRED	TREGACGICA	GACAACOOGO	-
	CCCAAGCTCG	GOOCATAGGC		CACTACCTAC	GCCANTITATIO	GACCACAGG	TICKETICCAC	ACCETOCAGE	CHGTTGCCCC	CTCACCACGA
10301	THOCTHOE	Trechoode	OCCOOCTOCT	GCGCTAGCTT	THYTCHCCAC	-	CMACCEANGE	COTTACOCTO	GANACICIANA	GCATTANGT:
	ANCCOMOD	MODIFICE CO	CCCCCCACCA	CCCCATCGAA	MACCOSTO	Ancorotation	GICCCATTCO	CCANTCCOAC	CHICOCIFI	COTANTICAL
10401	acreactee	+OTACCCOA	OCCUTATION	_	-	-	CACTETEOGA	CCTGCCGGAC	TOCOGCOANC	GGGGGTTTG
	CONOCOVOO	ACATCOCCCT	CCCAATAAAA	CONTROCCARC	TCACCCCCT	COCCANG	CTCAGAGCCT	GCCGGCCTG	ACCEPTATION	רניניאאייי
10501	CICCCCOICA	TOCANGACCC	COCTTOCAM	THECHECKER	-	_	TIGGITATICS	CAGATOCATO	COSTOCTOCO	
	GAGGGGGCAGT	P ACUTICACOO	OCCUNCTIT	AAGCAAGGCCT	THEMOCOTOC	TCGCGGANA	MCGMAAAGG	GICTACGING	GCCACGACGC	
10901	CCCCTCCTC	: AGCAGCGGCA	AGAGCAAGAG	CAGCGGCAGA		-	CCTCCTACCO	COTCAGGAGG	GOCGACATCC	GCGGTTCACT
	COCCOMOCAG	TCOTCOCCOT	Terestricie	CICCCCGICT	GIACCEG	TYSONGARA	GUNGGATAGC	OCAGICCICC	CCGCTGTAGG	
10701	COCCADENDA	1 TOOTOATTAC	GARCECECOC	DECUCCION			ACICUAGOCCOA	0000010000	COOCTAGGAG	
	accencence	P ACCACTANTO	CTTGGGGGG	ככשכעמעכבבנו	GOCCOTOATO	CACCTGAACC	TYCTCCCCT	CCCCAGNCCOC	OCCUPITECTIC	
10801	TOMOCOCCAC	: CCANDOTTOC	AGCTITANGCO	TOATACGCGF		-	GARCETGETT	COCTACCOCO	AGCGAGAGGA	
;	ACTEGECONO	DOTTCCCACO	TCGACTTCYC	ACTATOCOCA	CHCCGCATGC	ACCIOCOCOCO	CTTCCCACAAA	(acachoacac	Tecenerica	
10901	ATCCCCCATC	GAAAGTICCA	במכאישמננמנ	GACCTCCCAC	ATCCCCTTANA		THETRECACO	AGGAGGACTT		
1	TACCCCTAG	: CHITCAAGOT	-	CTCCACCACC	TACCOCACTT	AGCGCTCGCC	AArgardoog	TCCTCCTGAA	ACTEGOGET	Hadia Hadia
							CEETTEAMOREA	CORCATTANC	TTCAAAAA	THICAAAAA GCTTTAACAA
11001	CUATTACTCC	c cacacacacac	CACGTOGCTRO	CCCCCCOACCT			CCCACTICAT		AAAGTTTTT	CCIMANTTRITI
:	CCIMAICAG	٠.					CTTTTTTANGE	GCGCTOGAGC	MAACCCAAA	TACCAACICCG
11101	CCACCACACAC	TOCOMACACC					GNANCATTCO	CCCCACCTCO	TITORACITI	ATCGTTCGGC
11201	CTCATOGCG	C MOCTOTTICCT								
	GAGTACCOCC	TCCACAAGGA	ATATCACGTC	GICHCGICCC	ופדימכויכת	TANGTCCCTA	COCCACCATT	TOTATICATICT	COCCENCICE	מכמערכנישרה

AATCTEACT GAAGTITCT CTTCCACG/ CTTACACG/ GACTIACOTIT GCCTACTICG CGGCTACGCG TAATTACACA ACTCATOCTC	AGCCOTCCP: TCGGCAGGC' GGCCAACCCP: CCGGTTGGC'	MODECATER TECENOTRA TECENOTRA ACACCCCCT GEORGEANT	GCCCCCTCTCT	CCACAGOCIA GORCACATAC CCTGTGTATA GOCCTAGOGG CGCGACCCCG	ACCICCTO(T) CATCHTCTTOS CATCHTOCTTO GTACCTTOOT
	CTGCAGAUCC AGGACGTCTCGG TCAGCAGCCGCA GGTCGCCAAACCGGCA GGTCGCCAAACCGGCAAACCGCAAACCGCAAACCGCAAACCGCAAACCGCAAACCGAACCAACCGAACCGAACCGAACCGAACCGAACCAACCGAACCAACCGAACCA	GGCCGAAAAC MCCCGACTITTG TCCCGACCGAC TCGGCCGAC TCGGCCGAC TCGGCCGAC TCGGCCGACCGACGACGACGACGACGACGACGACGACGACG		CACOCCCAAG CCUTACTCCG CCCTACAGGCC TOTCAGCCGC NCAGTCGGCGC	
COCCATCAC 1 CCCCATCAC 1 ANGATUTACO OCCAGCTCAC CCCTCACCC ACCACCACCC CCCTCACCCC CCCCTTATAC CCCCTTATAC	GCGCGGCGC GCGCGGCGC GCGTTCCGGC CGCAAGGCCG	ATTTGCGCGA GCNGACCAAC CGCTGGTTG AACGCCTTGC	AMOTOROGE TITCACTCCA	CCTCTOGGGG CGACACTCCCC CTCTCACCGT AGATTACAAG TCTAATGTTC	ARATECECTE OTTGEACHT TTAMACAGGG TETAXXXIAG CAACGTGTCA AATTTGTCGE CAACGTCGAG CAGGAGATGA CCGGGCGGAAA GTCACACAGC GACCTTGTACT GGCGCGCGTT
	ACCCRRCORT TREGECTRICA CAATECTRAC OFFAGGMETS		CCANCOTANT GAGACACCOC CTCTGTOGCO	TRIANCETCC GLYCTTCACG CTATANGTYC ACTTCCAGG	
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Figure 15H 25/144

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12901	CICATOTATO	CCTCANACCT	OCCUPTANC	AACTUCTAA	TYXIACTT	CANTARTATA	ULLUSTED STATES	ACCCCCMOTA	TTCACCAAT	CCCATCTTCA
	CCCTACATAC	COAGITICOC	COCCAANTAG	TTATATO	ACT'T':ATCAA	CHINATA	このこのこのことと	TYSTAXCAT	MANDEGETTA	CXTACUACT
13003	ACCCUICACTO	GCTACCOCCC	ECTAMPACT	ACACCTXXXX:	ATTRICIACY:TY:	CONTRACTOR	ACCATURANT	CCTCTCOGAC	GACATAGACG	ACMENTAL
	TOCOCUTOAC	CGATCCCCCC	GGACCANAGA	Transcence	TAAGCTRYCAC	CHARTERIAL	TKACTACCTAA	CHAGACCCTO	CTOTATCTGC	TOTOCCACAA
								FüreNil	•	
13101	TICCCCCCAA	CCCCAGACCC	TOCTAGACTT	CACAMERICA	CAPCACACTOCAG	MAXICACIAL	GCCAAACCAA	GCGAAAGGAA AGCTTCCGCA	GOCCAAGCAG	CPRGPCCGAT
	AGOGGCOFF	COCCIETOSO	ACCUATICITION	CGITCICCCC	CHICHARITE	Trucksconich		CUCTITICCTT TCOMOCCOT	CCGGTTCGTC	GAACAGGCT?.
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13201	CTACCCCCTC	COCCCCCCC	OTCABATOCT	ACTACICCIAT	TTCCANSITT	TTCCANTITT GATAGETTET	CTTACCARCA	CTCCCACCAC	CCCCCCCCCC	CTGCTGTGC:
	DATCCGCGAC	OCCODODCOC	CAGICTACGA	TCATCOCCTA		AAGCTTCC:NA CTATCCCAGA	GAATGOTCGT	GACCOTOGIO	000000000	GACCACCCC
			P. Commercial Commerci	_						
13301	ADCAGGAGTA	CETAMERAC		TCOCTRCIVIC MACCOCAGOG	CONNINNAC	CHUCCHOUGO	CATTFECCA	CAACOGGATA	GAGAGCCTAG	TOGACANIAT
	TCCTCCTCAT	COATTIGING	AGCGACGACG	TOGRESTICAL	CCTTTTTTB	CONCUMENTAL	GTAMODOTT	GTTGCCCTAT	CTCTCGGATC	ACCT: TTCTA
13401	GAGTADATO	-	COCAGGAGGA	CARGGACTTO	CCAMACTEGE	CUTCHECCAC	CCOTCCTCAA	AGGCACGACC	GTCAGCGGGG	TCTOGREPOR
	CICATCTACC	TICTOCATGC	DEGRECIPOR	GTCCCTGCAC	פמשו הראות	CHRONINGIA	GOCAGGAGT	recordence	CAGTCOCCC	AGACCACACT.
13501	GAGGACGATO	ACTOGGCAGA	CCACACTCACC	GICCIOCATT	TECHNICATING	TYCANCECTS	TITICCIACOC	TTCGCCCCAG	CCTCGGGGAGA	ATCTTTAAA
	CTCCTGCTAC	TOAGCCGTCT	GCTIGHTCOMEG	CAGGACCTAA	ACCENECATE	ACCEPTION	NANCINCTURE	AAGCGGGGTC	COACCCCTCT	TACAMATTT
13601	ANNAMA	GCATGATGCA	ANTANA	CTCACCAACK	CCATTACACC	GAGCTITITAGE	TITICITISTAT	TCCCCTTAGT	ATGCGGCGCG	COCCCATGTA
		COTACTACGE	TITATITIE	GAGTREOTTCC	CASTACTOTOS	CTYXCAACCA	ANAGAACATA	AGRIGGAATCA	TACCACCACAC	OCCUUTNCAT
13701	TOADGAAGOT	CCICCICCCT	CCTACCACAG	TOTOGRANICANSC	CACCACACACACA	TOTAL	CCTINOCITICS	CCCTTCGATG	Crecectoda	CCCGCCCTTT
	ACTUCATION	GOAGGAGGGA	GGATGCTCTC	ACACCACTCG	COCCOCOGIC	ACCCCCCCCC	CGACCCAAGA	OCCANOCTAC	GAGGGGACCT	GOCCGCCAA
		Roma								
13801	oraccrecoc	GGTACCTGCG	OCCTACCORD	CHEAGAMACA	CCATCCUTTA	CICTIFACTIC	GCACCCETAT	TORCACACCAC	CCGFOTGFFAC	CTGGTGGACA
	CACCOMOCOCO	CCATGGACGC	COGNICCICC	CCCTCTTIOF	CCITAGGCAAT	GATACTCAAC	CCTCCCCRATA	ACCTOTOGOTO	CCCACACATO	GACCACCTOT
13901	ACANGICANC	CONTOTOCCA	TCCCTTAACT	ACCMINACION	CCACACICAAC	TETCTICACCA	CONTRATTEN	MACANTOAC	TACAGCCCOO	GGGAGGCAAG
	TOPICAGING	CCTACACCGT	MOCCACTION	TESTETRICT	GGTGTCGTTG	NAAGACTERT	CCCAGTAAGT	THOMACHO	ATGTCGGGCC	CCCTCCCTTN
14001	CACACACACC	ATCANTCTTO	ACCACCOCATC	GCACTRIGRATC	CKICTACCTVIA	AMCCATCCT	GCATACTAAC	ATGCCAAATG	TOANCOAGTT	CANCILLAGE
	GIOTOTOTO	TAGTTAGAAC	TGCTCGCCAG	ניהופאכניכנס	CCCICTOGACT	TTEGGTAGIA	COTATION	TACOGITTAC	ACTITOCTICAA	GTACAAATA
14101	ANTARGETTIA	AGGCCCCCCCT	GATCOTOTOR	COCTTGCCTA	CTAAGGACAA	Transmichi	CTCIAAATACG	AGTGGGTGTA	OTTCACCCTC	CCCCANTAGA
	TTATTCAAAT	TecacacecA	CTACCACAGC	OCCUNCOCAT	CATTCCTGTT	AGTECACYTIC	GACTTTATGC	TCACCCACCT	CAAGTGCGAC	GCCTCCCGT
				ŧ	Pari			•		
14201	ACTACTCCCA	GACCATCACC	ATACACCTTA		TGAACAACCC CATCGTCKIAG	CACTACTEDA	AACTCXXCAG	ACAGANCOCO	GTICTOGAAA	CACCINCATICOS
	TOATOAGOCT	CTOOTACTOO	TATCTCCAAT	ACTIVITION	CTAGE:ACCTC	CITCATCANCT	THEACCOOPE	TOTOTACCC	CANGACCTTT	CACHIFFACCY
14301	COTANGETTE	GACACCCCCA	ACTTCAGACT	CONTITION	وزسطيرانهن	(FIX.TTK:RCAT	CCCTVXXXXTA	TATACARACO	ANGCCTTCCA .	TCCAGACATU
	CCATTICALA	CTOTOCOCCT	TOMOTOTON	CCCTANN.TI	CHATACTING	CHGANCAGTA	CONTACCIAL	ATARCHTROC	TTCCCANCCT	MASTICTUTAL
14401	APPLICATION	CACCOATCCCC	GGWEACTTC	ACCCACACAC	GICTIGACKIAA	CTTGTTGTTGC	ATCITICANGE	OCY, MACCETT	CCAGGAGGGC .	TTTAGGATCA
	TAMANCGACG	GTCCTACGCC	CCACCTGAAG	TOXITORIOG	COGACTORIT	משכשככנט	TARKCUTTCO	CCCTROCAA	GENERALCE	MATCCTAGT

Figure 15I

CHRICCEGE CCCCACGEG TRANCOREA TOCCATTON TRANCOREA TOCCATTON MCTTOCTAGE ACCCAGGO TRANCORE T	GATGACCAIC GATGACTACA GTGAGGTACT Asst TTTTGGCCCCT	AGRECAGEA TEAGTTCGET AAAACTTTTT ACCACACAC TEGTTGTGG GGTGTTGGG GGTGTTGGG GGTGTTGGG GGTGTTGG GGGGGG
CTRITCEGE CTRITCEGE CTRITCEGE CTROCTAGE ACCEGAGGE TGGGCTCCAG ACCEGAGACC TGGGTCATGG	CCCACCOTCCA OTTCCCCTC CAACCOCCTC GAACACCACA CACTGOTCT	THEOGRAPH CETAGGRAD JACOGOTHET COTAGCTCC GOOTCCTNTC CAGCCOCACT COCHAGIANIA CTCGGGTTGA COCCOGNITC TTCGCGAGGT GOCCOGNITC TTCGCGAGGT GOCCOGNITC TTCGCGAGGT COCCOGNITC TTCGCGAGGT COCCOGNITCGG GOCCOGNITCGG GOCCOGNITCGG GOCCOGNITCGG GOCCOGNITCGG GOCCOGNITCGG GOCCOGNITCGG GOCCOGNITCGG GOCCOGNITCGG GOCCOCOGNITCGG GOCCOCOCOGNITCGG GOCCOCOCOGNITCGG GOCCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCO
	ACCTOCOCCT CTCAGGAGT TOGACCCCAG GCCTCGTCCA GCCCCAGGCT GTTGCCCGTG CGCGGCTCGA CAACGGGCAC TCGCTTTCCC GAGAACCAGA AGCGAAAGGG CTCTTGGTCT	
	ACTITATION FECTORICITY ACCIDIONOST COGNOCADOST ACCIDIONOST CONTENTENT TOGNOCOCOR DICTIONICOS CONTENTENT TOGNOCOCOR DITIONOCOCO GIOCOTTOGNOCA CANCOGOCOC COCOGNICOS CANCOGOCOCO TOTICINAMENTENA TOCOTTOCOC DADANGOCADA AGANDACITA AGGANAGOG CICTIONICOS	
GREPAREME C CREMENTE C CREMENTE A CRECENTER C ARTHREACTAN TRACAMETRA	TYCTTTRICAC CARCAACTTT GTCGTTGAAA TTCTGAACTC AGAGACTCGAAA	COCANTORCE GITCHATTOC GITCCATAT TTCCCANGA ANGOSTICAT COCCATTOR COCCATTOR COCCATTOR COCCATTOR COCCATTOR COCCATTOR COCCATTOR COCCATTOR COCCATTORCE GOSTIVORE COCATTORCE GOSTIVORE COCATTORCE GOSTIVORE COCATTORCE GOSTIVORE COCATTORCE GOSTIVORE COCATTORCE COC
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CCTRCGATUA TCTGGAGGGT CGTAACATTC GGATGCTACT AGACCTCCCA CCATTGTAGG TCCGCCGTCG TAGGCAGGG CCATGGTCG TCCGCCGTCG TAGGCACACA GGCGACACCT TTGCCACACA GGCGACACCT TTGCCACACA GCCGACACACCT TGCGACACAC TGCTAGGAGGGG TGGGAGGAGGGGGGGGGG	Kpn cerrocatac Methodoco guancotato Troatococo troccadica foatocado Accoreto Actacotec gettetada coacagoc	
14601 A 14601 A 14701 G	15001	15201 15301 15401 15501 15701 15801 15901

Figure 15J

16101		OCCCCOCA CIA	TCTATOOCC	CCCTANGANG	どりとしていることが	ATTACAAGGE	CTCAAACCTA	ANGCOORTCA	ANAGGANAA	GNANGATRIAT
	COTCCACTAG	COCCOCCTCT	AGATACCIOCI	OGGETTECT	CTT TY: GING	TAATK;TTC(X)	CONTRACAT	THUSCOCKAGE	THICHIT	CTTTCTACT.
								Sall		
16201	CATCATCANC	TROACGACGA	CONTRAMETO	CTRICACCICTA	כי ושישים יכיבאו:	CHICANTATA	CACTOCAMO	GINGACOCCI	AAAACOTOTT	TIGGGACCC
	CTACTACTTO	AACTOCTGCT	CCACCTTICAC	GACCHACGAT	CHROCOCOCCIN	COCTOCCO AT	GICACCITIC	CAGCTGCCCA	TTTTGCACAA	AACCCTGC:
16301	GCACCACCOT	AGECTITACO	CCCORTINAGE	GCTCCACCCG	CACTTACAAG	COCCURATANG	ATCAGGTGTA	CYCCGACTIALS	GACCTOCTTO	ACCAGGCCAA
	COTOGRADCA	TCAGANATGC	NGOCCACTOS	CGACCTGCC	GTCGATGTTC	CHYCACATAC	TACTCCACAT	OCCOCTOCTC	CTOGACGAAC	TCGTCCGGTT
16401	COMECUCIA	CHICAGOLITEC	CCTACOGNAA	(CCCCCATANG	CACATTACTAG	CONTRACTOR	GGACGAGTGC	ANCECANCAC	CTAGCCTAAA	GCCCGTAACA
	OCTOCOCOLO	CCCCTCAAAC	COATCCCTTT	COCCGTATTC	CTGTACGACC	GCANCOBCTA	CCTGCTCCCG	TICCOLLICTO	GATCOGATIT	COCCATTGT
	F									Kint
16501	CTUCAGCAGG	TOCTGCCCOC	GCTT OTACCO	TEEGAAGAAA	ACCICCIONET	AAAGTTEGAO	TUTOSTUNCE	MACACCCAC	CONOCAGETO	ATRIOTARICCA
	GACOTCOTICE	ACCINCUISCO	CCAACCTOGC	MGG:TTCT-TT	Tedegecons	Tricyactic	AGACCACTGA	ACCGROOOTO	GCACOTCGAC	TACCATAGGE
16601	MCCCCCAGCG	ACTUGAAGAT	GICTIGGAMA	MATCACCCT	GGAACCTRAX	נידואנאינכככל	AGGICCCCOT	OCCCCANTC	ANGCAGGTOG	COCCOOCACT
	TCGCGCTCGC	TOACCTTICTA	CAGAACCTTT	TTTACTGGCA	CCTTOGACTC	GACCTCOOGC	TCCMOCGCA	CGCCGGTTAR	TTCOTCCACC	OCCCCCTGA
16701	DODCOTOCAG	ACCOTOGACO	TTCAGATACC	CACTACCAGE	AGCACCANTA	THRECACCRE	CACASAGGGC	ATOGAGACAC	AMCGTCCCC	GOTTBCCTCA
	CCCOCACOTC	TOCCACCTOC	AACTCTATOO	CICATOCICA	TCCTCGTCAT	AACIGETOGCG	OTGICICCCS	TACCTCTOTO	THYOCAGGOG	CCAACGGAGT
16801	acaataacaa	ATOCCOCOOF	OCACIOCTOCIT C	OCTURORCO	CGTCCAAGAC	CTCTACGRAD	GTTSCALACTO	ACCCUTOGAT	OTTICOCOLI.	TCAGCCCCCC
	COCCACCOCC	TACOOCOCCA	COTCCOCCAG	CCACCCCCCC	CCARPITICITO	GAGATACCTC	CACGTTTRACC	TOCCACCTA	CANAGCGCAA	ACTCGGGGGG
16901	acceceaca	CCUPICGAGG	AMETACOGCO	CCGCCAGCGC	GCTACTGCCC	GANTATRICCC	TACATCCTTC	CATTGCGCCT	ACCCCCGGCT	ATCGTGGCT
	دحمحمموحمح	OCCAROCTCC	TTCATGCCGC	GOCCOSTICIOCO	CCATCACCOC	CTTATACGUG	ATCTAGGAAG	GTAACGCGGA	TOCCCCCA	TAGCACCGAT
17001	CACCTACCOC	CCCAGAAGAC	CAGCAACTAC	CCGACGCCGA	ACCALCACTG	CAACCCCCC	ככנאטינאנסככ	CETCGCCAGC	CCGTOCTABC	CCCGATTECC
	OTOGATOCCO	OCOTOTICE	CTCCTTCATO	GOCTGCGGCT	TROTOGICAC	CTTONGCOCC	GOCCACCACCG	GCAGCOGICG	OCCACGACCO	GOCCENANG
17101	OTCCOCHOOD	TOCCTCCCCA	AGGRAGGENOG	ACCUTOTOC	TOCCARCATO	CACOCTACCAC	CCCARCATEG	TTTAMANGCC	CONCINION	GTTCTTGCAG
	CACGCGTCCC	ACCOMOCOCT	recreessee	TOGGACCACG	ACCASATIGACG	COCCOATGGTG	GOCTOCTAGE	AAATTTTCGG	CCAGAAACAC	CAAGAACGTY
										Sphi
17201	ATATOOCCT	CACCHOCCOC	CHCCGTTTCC	COCTROCCOC	ATTICCGAGGA	AGANTGCACC	GTAGGAGGGG	CATOGCCOOC	CACGGCCTGA	COCCUANCAI
	TATACCOCCA	STOCACGGCG	GAGGCAAAGG	OCCACOOCCC	TANKICTOCT	TCTTACCTCG	CATCCTCCC	GTACCOGCCG	OTOCCOGOACT.	GCCCGCCGTA
	5 }				Sphi					
17301	oconconaca	CACCACCOOC	GOCOOCCCCAC	CTRISCACCOT	CCA, ATHRCFACG	CCCCTATCCT	GCCCCTCCTT	ATTCCACTOR	Trecretore	GATTIGGCGCC
	COCNOCACOC	orteoroacco	כבפבבפבפב	CAGCRINGCCA	GCCTACOCYC	CONCATACK	CCCASCACACAA	TAAGGIRINGF	AGCGGCGCCG	CTAACCCCCI
17401	GTOCCCOCAA	TTGCATCCOF	GGCCTTGCAG	GCGCAGAGAC	ACTITATION	AACAAGTTAC	ATICTIONARA	ATCAAAATAA	AAAGTCTOOA	CTCTCACGET
	CACOGGCCTT	ANCOTROCCA	CCGGMCGTC	COCCIECTOR	TOACTAATT	TRETTCARCO	TACACCTTTT	TATTTTOAT	TTTCAGACCT	GACACATOCCIA
				-						Econt
17501	COCTRODICC	-		THOTAGAATG GAAGACATCA	ACTIMINACIONE			CACACACAGIT	CATOGGAAAC	TRECAMENTA
	OCCUMECAGO	ACATTCATAA	MCATCTTAC	CFICTOTAGE	TCAAACGCAG	AGACCTARGAC	CCTGTGCCCA	GCGCGGGGCAA	CTACCCTTTG	ACCOUNT

Figure 15K

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Figure 15L

19301	ACAACTAATO	GOCCARCAT	CTATGGGGAA	CACACATANT	TACATIVATIT '	٠.				COSTAATATA
	TCTTGATTAC	CCCONTICITA	GATACGRETT	CITCLINIATIA	ATCTANCCAA	ANTECETISTE !	_	_	_	CCCATTATAL
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	TARCTICORC	CATGAAAAGA	TACACCTTAG	TOCCACAACT	CTTCCATAC TA	GATETACAAT (:	-	PCACTIN:TAC	TTCAA' X :T'T'
19601	TTACTOCTIT	CCACTCGGAG	GTCTGATTA	TACAGAGACT	CTTACCAAVS	ATTEROGATE 1	AACAGGTCAGT	CHITTACCTA	CCCTTTTCT	TOCTACAGAA ACGATGTCTT
10701	ANIGACONA.	BERNTIAAAT	AAGAGTTGGA	MTMTTT	CCATCACAAAT	_	_	_	CCHOTACTCC	AACATAGCO
10161	AAAGTCTAT	THEACTIFA	TTCTCAACCT	TTATTANAC	GGTACCTTTA	GTTAGATTTA (COGITOCACA	CCTCTTTAAA	CCACATONOO	TTGTATCGC:
19801	TOTATTACC	_	AAGTACAGTC	CTTCCAACGT	AAAATTICT	GATAGGCAA	ACACCTACCA	CTACATGAAC	ANGCCANTOG	ACCCACACACA
•	ACATANACOO	_	TICATORCAG	GARGETT G.A	VIII.00				GCAATGCTUG	CCTGCGCTMC
19901	CCTACTOCAL	ACCATCATTA	TOGATCCTCG	ACCCTURATECT TOTGACCAGO	GANCTGATAT	-		_	COTTACCACC	OGACGCGAFM
20001	CENTRANTER			GROCCCTICC	ACATTICAGET	GCTCAGAAG	PICTITICE		CCFTCFCCTO	CCOGGCTCAT
	GCGAGTTACA	_	ACCAGCGATA	CACCGGGAAGG	TOTARCTCCA	COCAGICTIC	MCMACGGT	AATTTTTGGA	GGANGAGGAC	GOCCCCMCTA
					Pstl					
20101	ACACCTACGA	GROOMACTIC	ACCANCIANTO	TTAACATOGT	TCTTTACAGE	TECETAGGIA	ATOACCTAAG	COLLONCOCA	OCCAGCATTA	ACTITICATA:
1	TOTOGATUCT		TCCTTCCTAC	AATTOTACCA	ACACTITETE	AGGGATCCTT	TACTOGATTC	CCAACTGCCT	COCICCIANT	TCAMCTAN:
20201		TACCCCACCT	TCTTCCCCAT	GGCCCACAAC	ACCIOCITECA	CGCTTGAGGC	CATOCTTAGA	AACCACACCA	ACGACCAGIC	CTTTANCO!
1	GTANACCOM	•	AGNACIOCITA	CCCCCTGTTG	TCXXCXCACCT	GCGAACTCCG	GTACGAATCT	ricciencer	TGCTGTTCAG	CAMPITOL:
20301	TATCTCTCC	CCCCCAACAT	acretiaceet	ATACCCGCCA	NCTCTACCAA	CONGCCCATA	TECATECECT	CCCCCAACTO	OCCCCTTTC	cocoscions
)))	ATAGAGACCC	: GOCGOTTOTA	CCACATOGGA	TATEGOCCOT	TOCCATOOTT	CCACCACTAT	ACCTACCCCA	GOCCUTICAC	CCCCCCAMA	פרפררושוייי
20401	CCTTCACCC	CCTTANGACT	PAGRIMANCCC	CATCACTGGG	CTCXXXCTAC	GACCCTTATE	ACACCTACTC	TOCCUCTATA	CCCTACCTAG	TATGGGGGGGGG
	CONNETCCCC	: CCANTICTOR	Treetings	GINGICACCC	GAGCCCGATT	CTGGGAATAA	TCTCCATCAG	ACCORDANA	Godni Conic	
20501	TRACETICANC		-	-	GACTCTTCTO	PCARCTICACC AGENTALITY	TCCCAATCAC ACCCTTACTO	CCCCTCCTTA	CCCCCAACUA	CWACTTTA
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20601	MOCATICAG	THEALTHCACH	r cccwarento		CATTGTACTG	OPPTICACC	AAGGACCATG	TITACCATICG.	ATTGATATTG	TMCCCGATUR
נמכמכ	POST ALL ST	•	_	ACCCATGTA	CICCTICITY	AGAMACITICC	AGCCCATGAG	CCOTCACOTO	OTCCATCATA	CTANATACAA
70103	TCCCGAAGAT	_		•	CACCAACAAA	TCTTTCAGG	TCGGGTACTC	GOCAGRICCAC	CACCTACTAT	CATTTATOTT
20801	GGACTACCAA	_	-	ACACAACAAC	-	TTGGCTACCT	אסכונכנלאכנ	ATCCCCCANG	CHEMICHERY	CCCTICCTAM
	CCTGATOUT	r onccaeced	r Aggangtogg	TOTOLINA TOTOLINA	AGACY, TANAC	MUCHICAN	Pref			
20901	TECCETATE	C COCTTATAGG	3 CANGACCIACA			AAAATTICTI	TVCCRATCOCA	CCCTTTGGC	CATCCCATTC	TCCAGTAACT
	AAGGGGATAG	3 OCGANTATOC	c crichocci	CAACTRATEGE	AATGGGGTCTT	TTCMMGAA	ACALTMOCOT	Klaverner		

Figure ISM

COUNTRY COUNTR	INCT INCA INCA INCA INCA INCA INCA INCA INCA	PAC. NIGH GAAV. CTM '	CACT GTK1 ATTAA TANTT	CCAC!	ICAGO ICAGO AGREC AGREC	NAGACTT TTCTGAA	GINCITICACG CINCIANGTUC
CCATGGACGA GGTACCTGCT CACGCCCTT : GTGCGGGA !	TCANAGNTCT AGTTTCTNGA GGCCGGTCT> CCGGCCAGTC3 CGACTCAACC GCTGAACCCCAACCCCAACCCCAACCCCAACCCCAACCCCAACCCC	AAAGGGTAC. v FFICGCATGF CACCATGAN' GTGGTACTT '	CHCGCGGTG CTTTCARTAN GAAAGTTNI'' COCATCGCTN	OCGTAGCGAT TCACTCCACA AGTGAGGTGT		ACKROCACRO COUNCIACITY . TICTGAA	CNC
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CATCACTTT G GTACTGAAAA C ATCGAAACCG T TAGCTTTGGC A	GCNGGACTG A CUTCCTTGAC 1 GCCTGCGCA 1 CCGACGCGGT A CCTTTGGCTT 1 GGAAACCGAA A	AACGCTGGAA TIGCGACCTT 1 ACTCCCATGG 1 TGAGGGTACC 1	AACAGCTCTA TTGTCGAGAT AAAATAATGT TTTTATTACA	THE STATE CONGROUS GEOGRAPH COCCOPICA CONTINUATION COCCOPICAN CONTINUATION CONTINUA	TRANSTICETCE GECETIGGEG CRECIANTES ACCECTIONES COGRACIGES GENETICANO CRIMINATA TECNOCATECA GENETICEGE CENTRATET ANGENICATOR CANCINATION		
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GOOGGCACTC P CCCGCOTGAG CTTTATOTTT I		COCATGRONG GRETIONGTRC CAAACTCATG TCGGCCGCT ACCCGCCGA	Kpm GOOTACCCAA CCCATGGOTT CCGCAGCCAC GOCUTCGGTG	######################################	CATCACCIAC GTAGTGGTTG TGGAACACTA	TCAACTTTOO AGTTGAAACC GTTAGGATAC CCATCCTATO	C TGATTGGCC
TTATOTCCAT 6 ANTACAGGTA C GCCCACCCTT CGGGTGGGA		CTCTGACCCC AGGTTTACCA TCCAAATGGT GGGCCCCAAC CCCCGGGTTG	CHIATIACCE GAATAATGGC CGCCCTACTT	ACCCATANCE TOCCOTTTACE TOCCCACTE ACCCOTCAC	GOCTGCGCAC CCGACGCGTG GTTGCAGCAC	CCCANCOCAC CCCTTCCCTC CCCTCCCTC CCCACACCCCC	OCCOGNAMA COCCCTITI
21101	21201 21301 21401	21501	21701	21901	22101	22301	22501

Figur 15N

PNRKACISGAY MER682

10966	W. A. S. C. A. Market St. W.	Tractalities	CHEST PROPERTY.	CCCCCATICATE	CONTRACTOR	CONTRACTOR A	ATTICANTON (CONOCICCITY /	ATTTATCATA 1	ATGCTTCCGT
10077	TREATTORE		GACHTANTATE				TANASTINGT (GCACGAGGNA .	TAMATAGTAT '	TACGNACKICA
										<u>?</u> }
10266	CT SCAPE STATE	A brankring	Triban III. NG	CCCAGCGTT	CWECACAAC	מימיאמככרת יו	TOCOCTUCTO 1	ATCCTTCTAG (GICACCTOTO	CNANIGACTO
3			ACCTACACIC					TACGAACATC	CAGTOCAGAC	GTTW:CTX:A
	15.6	P. 25								
22801	CAGGIRACGC	TOCACCANT	OCC.CATCAT	CCTCACAAAG	GETTOTAGE	TEXAMENTA	CAGC'PRICARC (ccocooract	CCTCOTTICAG	CCAGOTCT1.1
	OTCCATGOOD		CGCCGTAGTA		CARMICANCO	ACCACTTONA (GTCGACGTTG	GOCOCCACGA	GCARCAAGTC	GITTCAGAAC
22901	CATACGGCCG		CACTTCX:TCA	OCCAGINGIT	TEAMETTICE	CTTTAGATED '	TTATCCACGT	GGTACITICIC	CATCACCGCG	CHACHACAGCTT
	GTATGCCGGC		GTGAACCAGT		ACTICARGED	GNANTCTACE !	NATAGGTGCA	CCATGAACAG	GTAGTCGCGC	OCOCOTICO 1
			Prof							
23001	CCATGCCCTT	CHCCCACGCA	GACACCATUG	GCACACTCAG	CGCATTCATA:	ACCOUNTABLE O	CACTITICCOC	TECKTOOR		CCICITACCAT
•	COTACOCOAA		CTCTCCTAGC	corcranate	OCCCANGTAG	TOCCATTANA (CTGAAAGGCG	NACCONCCCO	MONACCAGAN	CCCACACCC
21101	CCCCATACCA	COCOCCACTO	OCHECTIC	ATTCACKCCC	PECACTOTAC	GCTTACCTCC	THURCANOC	TTCATTAGCA	_	GCTGAMA CICC
	OCCUPATOOT	_	CCAGCAGAAG	TAMETERIORS	GCGTGACACG	CONNTRICATO	AAACGCTACG	AACTAATCOT	OCCCACCCAA	COACTITION
23201	ACCATTIGIA		TICICITICS	TCCTVXXXTGT	CCACGATTAC	CICTOSTGAT	GOCGOOCGCT	COCOCTICOC	MANAGOCOC	TECTIFICE
1	TOOTAAACAT		ARGAGAAAGA	AGGACTICACA	OPPOCTANTO	GASACCACTA	CCGCCCGCGA	GCCCGAACCC	Terreceded	ANGALANAGA
21101	TCT-TOOOCOC		Tecaceacca	AGGEOGRAFOO	CONCORNER	CONTRACTO	GCACCAGGGG	GICTIOTORY	GACTICCE	CONCCINCOLA
	AGAACCCGCG	-	AGCCGGCGCC	TCCMGCTACC	BOCOCCCCOVC	CCACACCCCC	corocrocco	CHCIANCACTA	CTCAGANGUA	GCAMMAGCCT
(UPLC	CHUNATACIDE	COCCICATOC	OCTIVITION OF	GOOCGCCCCC	CHACKCACC	CCCACCOCCA	CHARAGACGAC	ACOTOCTOCA	1001100000	ACCTCCCCT.
1	CAGCTATGCG	_	COMMANDE	CCCCCCCCCCC	CCACCOCCCC	CACTGCCCCT	OCCCC:10CTO	TOCAGGAGGT	ACCANCCCCC	TOCAGOOC
21501	CACACTORIC		GOTGOTTCO	COCTOCTCCT	CTTCCCGACT	GRECATTICE	TTCTCTTATA	GOCAGAAAAA	CATCATOCHO	TCAGTCGM:A
7	CCTOCCCAG	_	CCACCIANGC	_	GAAGGCCTCA	CCCCTAAAGG	ANGACIGATAT	ccorcentra	CTAGTACCTC	AGTCAGCTC'
21601	AGAAGGACAG		CCCTCTGAGT	TOCCCACCAC	CULTICCACC	GATCCCGCCA	ACCCCCCTAC	CACCTTCCCC	OTCORDOCAC	CCCCCCTTGA
	TETICCTOR		GOGAGACTCA	ACCCCTCCTG	GCCCACACACTIVAG	CTACGGCGGT	TRICOCOGATO	OTOGAA0000	CAGCTCCCTG	COCCCCANCT
10716	AND PROPERTY.	_	ACCACCACC	AGGILLITETA	ACCGAAGACG	ACCAROANCE	CTCAGTACCA	ACAGAGGATA	AAAAGCAAGA	CCAGGACAN'
10179	CURCUETT		restectode	TCCANACAT	TCCCTTCTGC	TOCTCCTGGC	GAGTCATOOT	TOTOTOCTAT	PHYSOTHER	CONCENCIANS
,										
10000	Agreement of the second	Armaranta	Activities	GEGGACGAAA	CCCATOCCA	CTACCTAGAT	CTOCCACACO	ACGROCAGIT		CAGCGCCAUT
10067					CCGTACCGCT	GATGGATCTA	CACCCTCTOC	TREACGACAA	CTTCGTAGAC	GPCGCGGTCA
10016	Crant a Free	_	TTGCANGAGC	_	RECECTORIC	ATAGCOMATO	TCAGCCTTGC	CTACGAACGC	CACCTATTCT	CACHOCOCOCT
1000	CTECTETARTA		ACCITICAC	COTCOCTACA	CTATACACAC	TATCGCCTAC	NOTICOGRANCO	ватосттосо	GTGGATAAGA	OTCOCOCCA
10000			Acceptance	CHACCECANG	CCCCCCCCC	ACTICIANCE	CUSTATTRICC	GTOCCAGAGG	TCCTTCCCAC	CTATCACATE
70057	Transaction.		TOCCUTOTAC		COCCCCCACT	TCAACATGGG	CCATAAACOO	CACGOTOTOC	ACGRACCOTO	GATACHCITA
										A COMPANY
14101		AB ACTIONARY	ACCCTATCC		TREETHECEN ACCREAGES	ARCGRACANG	CAGCTCCCCT	TOCOCCAGGG		CCTUNTATO:
4215	AAAAAGG	TGACGTTCTA	TOGOCATAGG		TGACGACAGC	rescensine	GTCGACCGGA	ACRECENTER	GCGACAGTAT	CCACTATARY.

Figure 150

PMRKAdSgag MERGRZ

AACAGCGANA AYGAAAGTTA YYGYCOCYTY YACYYYCAGY	CCTACCCOC ACTINACCTA GOATGGCCG TAANTTGAT AGAACAACA GAGGAGGCT TCTTGTTGT CTCCC(*)		CCTFTCGACA GOGCTACGTA GGAAAGCTGT CCCGATCCAT				GANATCCTTG ANTWINSON CCGCTTTGGG GCCACTGCTA GGCGANACCC CGTTGACGAT	ACTORCOCTO CAACCTATKE TGACAGCGAC GITGFATACH	GACCGARTA GACCGARTA GACCTCAAT TCAACAAG ACTGTTTCG GCCCTATCAC CCCTATCAC
CCANCAGGAA P	ACCACTITO TOXING CONTINUES CANALITICS CONTINUE		TTGCACTACA	ACCOCCTTGG TOOCOGAACC	GACGGCCATG		ACAACCTTT ATGCCCTCCG TACGGGGGC		TOCAGOOTICS A CTACCAGO A TTACAGOOTICS A TTACAGOOTICS T ANCOTICOST C COCCCCCCC G GCGCCCCCCCCCCCCCCCCCCCCCC
	CATCEAGHTC GTAGCTCCAG GAGAGGGATG			-	ACACCTOGCA TOTOXIACCGT	GACGGCCTTC CTCCCGGAAG NOTCAAAGCA	TCAGTTTCGT A AGTACCGCGA T TCATGGCGCT	NTANTOGRAG ACOTGAGGG TYACGGTCTA TATTACCTTC TGCACTCTCC ******************************	ANTIMICATE ACCTITIONOC THANTAGECA TOGANICTEG CREANITION ACCTIONOGA GOSTITIONOC ATMANTECT ACTOCIONITY ACANCEGOT CRACECTON CONTECCE CRACECTON CONTECCE CETECONSTIG GOTTNOCAGE
ANGRESTEG CAAACHETET TTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG		CCTGCCGACT	CCARINANTIC ARCATANGET GACCITCIACO TCGCOTICOA	CTCHOCANCE TRESTCTCCTA CCFTRICAATT GAGACGTTOG ACCAGAGAT GGAACCTTAA	TTTCTATGCT AAAGATACGA	ACCIDENTAL TEXTEGRATAC STACTTECT		S ACOTGAGGGG C TOCACTCGG	
ACGCCCACCGGG	CTANCCCTAC GATCGCATG TRICGCCTGC	ALCATORIAGE ACTORIAGE TEXTS CONTRACTORIAGE TEXTS CO		TRGICICCTA	CGTTTACTTA	CANANCTIGA GITTIGAACT		C NTANTGGAAG G TATTACCTTC	A ACCANAGTICA T TGCTTTCAGT C CRCAATGGAA C CTCAATACCT G CATTAATACG G ATTCCGGTGG
AGGGTK TING TCCCAGAAGC	CNACIACATIA GETRATIKIALI GARETRIATER	CTCGNCTARC GCTGACTTCA CGACCGAAGT	CTTTGCTAAC	CTCTGCAACC	COCOACTACO TEROCOACTO GEOCIFIATO AGGEGETTAC PSII	ACTRICTARAGE TO THE TOTAL TOTA		A CCACTCTGAC F GOTGAGACTG	C CRUCINCOANT C GREGACOANT C TORROACOTC G ACACCTGCAO T PACCACCTTC A ATGGCGGANT C TRISACTCCC G AMICTICAGGG
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COANGTRICCA	TTGGTGGAAC AACCACCTTG TCATGAGCAC	AGTACTCGTG TOGCGACGAG ACCGCTGCTC		88	CONGOCOCOCO COCOCOCOCOCOCOCOCOCOCOCOCOCOCOC		A AGGGCTTGC ANTCTTGCC TTAGAACGG	CCTTCTGCNG CTAGCCIACT GUARGICGTC GATCGGTTGA	C GCTCCCTOGF G GGAGGGACCA G GTTGAAACTC C CAACTTTGAG G CGCCCGCTA G GCGGGCGAT C GAAAGGTACG G CTTTCCTGC
CCTCGCTCAA	CTCTGGAGTO GAGACCTCAC CCCCCCAAGG	OGGOOOTICC TACCCCCAGT ATCOCCTCA	#ACCGTGGAG ATGCCACCTC	COCCAGGCCF	CECTCAAGO	OGROGRAFICE CCTCCTCACO	GACATCATTT CTGTAGTAAA AGCGCTCAGG TCGCGAGTCC		ACCCCGGAC FEGGGGGGGG CCGAGGCCC ACCCAACC FCTCGCTAGG TTTCTGCTAC
24201	24301	24501	24601	24701	24801	24901	25001	25201	25301 25401 25501 25601

Figure 15P

PHRKACISgag Nergb2

1,20

25701	GOOCCETTUC		CICCACCT: NA	AAGAACTIC		שיגענגעיפ	האהמאממאמ	MEACTERICA	CAMPCAGGCA	CAGATACATTT
•	CCCGGGACG	Addoortecta	CCONCRIMEN	TTCTTCGACG	Trythyropes	CINTRACTURE	CHICHCHECTEC	TTATCACCCT	STCACTCCGT	CHICCHICCAN .
						Haville				
25801	TOCACGAGGA	GCACGACGAC	ATGATGGANG	ACTRIORAGAG	נינידאמאנימאט	GAAGCTTCCG	ACCTECTARGA	COTOTCACAC	GAMCACCCOT	CACCETTOO!
	ACCTOCITO	CCTCCTCCTO	TACTACCTTC	TGACCCTTTC	CONTETECTS:	CITCGMGGC	TCCARCTICT	CCACAGICIO	CTTTCTCCCA	GTCXT:AGC! A
25901	CGCATTCCCC	Traccoacac	CCCADANATC	GCCAACCCGCT	TECAGEANTE	CTACAACCTC	CACTCCTCAG	0000000000	CACTGCCCGF	TOROGRACCE
	OCOTANGOOO	AGCGGCCGC	COUNTING	CCCNTYRCCCA	NOTITICATIVE	מאמתוניות	GCGAGGAGTC	crecessori	OTCACCOGCA	ACCCCCTCCC
26001	ACCOPAGAT	-	TOGANCCACC	OCCUBITANGE	CCAMICAGCC	CCCCCCTTA	CCCCAAGAGC	AACAACAGCG	CCAAGOCTAC	CIRCICATOR.
	TTOOCATCTA	cccrotocns	ACCITIGGICC	CGCCATTCA	GOTTCOTCOD	CRECIPICANT	COCCINCTO	TIGHTORCOC	GOTTCCOATG	OCCUPATACCO;
26101	OCCORCACAA	GAACGCCATA	OTTOCTTOCT	TOCANDACTO	TOORGGEAR	ATCTCCTTCG	CCCGCCGCTT	TETTETETAC	CATCACORCO	TOOKS THEFT
	COCCCOIOLL	CTTCCCCTAT	CHACGMACGA	ACCITICTION	ACCCCCATE	TAGACICAACIC	GCCCCCCCAA	AGAMGAGATO	GTAGTGCCGC	ACCTRAACE!
26201	CCOTABCATC	CHOCATTACT	ACCORCATCT	CTACAGCCCA	TACTRICACES	GEOGRAPOCO	CAGCAACAGC	ACCOCCACA	CAGAAGCAAA	CACCIACCOGA
	OCCAPTICTAG	GACCITAATGA	TOCCAGTAGA	GATOTOCOGIT	ATGACGTYGC	CACCGTCGCC	gregrinates	recessorer	GICTICOTIT	CCACTOGCCT
26301	TAGCAAGACT	CTCACAAAOC	CCAAGAAATC	CACAGCGGCG	GCARCARCAG	CACCIANTANGE	ecrecercia	GCGCCCAACO	AACCCOTATC	GACCCGCGAG
	APCOTTCTGA	CACTOTITICS	OCTIVETITAD	GROTOGCCOC	COTCOTOGE	CTCCTCCTCG	CGACCCAGAC	cacagarrac	TTGGGCATAG	CTOGGCGCTC
26401	CTTABAAACA	GCATTITICC	CACTUTATAT	CCTATATTTC	AACAGAGCAG	GOCCANONA	CANGAGCTOA	AAATAAAAA	CAGGICTOTO	CCATCCCTCA
	GAATCTTIGT	CCTANANGG	GTGAGACATA	CCATATAAAG	THENETECTIC	CCCCGGTTCTT	GITCTCOACT	TITATITIE	GICCAGNONC	OCTACOCART
26501	CCCCCAGCTO	CCTOTATCAC	NAVGCGAAG	ATCARCTTCG	GCGCACGCTG	נואטאטטכפפ	MACCICITY	CAGTAAATAC	TOCOCOCTOA	CTCTTANGGA
	GOOCGINCGAC	DCACATAGTO	friresence	TAGTCCAAGC	CCCCTACCCAC	CTTCTGCGCC	TCCGAGAGAA	OTCATITATO	ACCCCCCACT	GAGAATTCC .
26601	CTAOTITICOC				CONCACHO	AFFCORCCACA	CCCGGCGCCA	OCACCTOTAG	TCAGCGCCAT	TATHINGCAN
	CATCALAGED	CODGRANGAG	TITARATICO	CCCTITITION	CCACTACAGG	TCCCCCCTGT	OGOCCACOOT	CCTCGACAAC	AGTEGEOGTA	ATACTCOTTC
26701	GAMATICCCA			CACCCACAAA	TGGGACTTGG	COCTGOAGCT	OCCCANGACT	ACTCAACCC	AATAAACTAC	ATGARCGCC
	CTTTAAGGGT		CACCTCAATG	GEOGRAFIE	ACCCTGAACO	CCCACCTCGA	COCCITICA	TCAGTTGGGC	Trafficato	TACTCGCGCC
		Eooftv			₩ 4	Econi Contractor				
26801	GACCCCACAT	GATATCCCGG	STCANCISONA	TACOCOCCCA	CCCIANACCCA	CCCAAACCCA ATTCTCCTCG	MACAGACACC	TATTACCACC	ACACCTCOTA	ATMACCITIAN
	CHOCOCOTOTA	CTATAGGGCC	CAGITICCCTT	ATGCCCCGGGT	CACTITIOSCI	TANGAGGACC	THETECOCCO	ATAATOOTOG	TOTOGAGCAT	TATTGGAATF
26901	PCCCCOTAGE	TOCCCOCTO	CCCTOGROTA	CCAGGIANAGE	CCCCCLCCCA	CCACTCTOGT	ACTITICCIONGA	GACGCCCAGO	CCGAAGTTCA	GANTACTAN
	AGGGGCATCA	ACCORDICAR	GGGACCACAT	GOTCCTTTCA	CXXCCCACGCTT	OTTRACACCA	TCANCOCTCT	Creceogree	GCCTTCAAGT	CTACTGATTO
27001	TCAGGGGCGC	AGCTTGCGGG	COCCUMICCT	CACAGOGIAC	ממוכהככנה	GCAGGGTATA	ACTICACCTOA	CANTCAGAGG	GCGAGGTATT	CARCTCAACT
	AGTCCCCCCC	TCOMCOCCC	GCCGMMGCA	OTOTOCOA CG	CCANCIBORCE	CCTCCCATAT	TOAGNOONCT	GETAGRETICE	CCCTCCATA	GYCGAGTITA.
27101	ACCADICACT	GAGCTCCTCO	CFTOOTICTICC	GTCCCGACCED	GACATTICAG	ATCGGCGGCG	centeractic	THEATTHEACO	CCTCGTCAGG	CANTECTANC
	TRETCARCEA	CTCGAGGAGC	GAACCAGAGG	CAGGCCTGCC	CTGTAAAGTC	TAGCCCACCAC	GGCCGGCGAG	AAGTANGTOC (GCAGCAGTCC	GITAGGATIG
	E									
27201	TCTGCAGACC		AGCCOCCCTC		GGAACTCTGC	AATTTAITGA	GGAGTTTGTG	CCATCOGICT	ACITITAACCC	CITICICOGRA
	AGACGICTOG	AGCAGGAGAC	TCOCCCCAG	ACCTCCOTA	CCTTGAGACT	TTWATMCT	CCTCAMCAC	CCTMCCCAGA	TGAAATTGGG	GNACAGECET

Figure 155

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GCAGAGCAAC COTCTCTTT ATATCGAGGG TATATCGAGGG GGACAGGGG GGACAGGGG CCTGTCCCTT	AGNATAN: A TETTTAAT! ATETETEC ' TAGAGAGG: A	AGGNATGRAC TSTTTACCAG ACAMTGGTC CTATTCTAAT	GCCTGCTU 1 CCCGACGCAC GTCAGCCCAC	ACCACAGA/ TGGTGTCTT ³ TTACAGTTT ⁷ AATGTCAAAA	GTOJCCCCCA CACCGRONG ATGITITIC "A AMONATE" "A THOTTAN "T ATANTOCKO "A
AMOTICINADO GO TTCACCTCTC CO CCCCANGGATC A GGGCTCCTAG T TAGTTCAGCC G	TAATAAATAC ATTATTATTATTATTATTATTATTATTATTATTATTATT		MOGETCOCC TECCOACCOC TECCOACCOC		AGNINAGIT TCATATACA GATATATATA GACTACCA GACTACCA TCACTOCO AGNINGACO AGNINCACOC
		-	TTANGTTCGT TTCTCTCCCT NAGAGGGGA CTAGGTTTAG	GCACCACTCT CGTGGTGAGA TGACACTACA ACTGTGATGT	ATENGCAMC TACTCENTTO CATGOGATON GGTHTACCO ACANTONOCT TTOTTANCTO
GACGGCTACG ACTGAATGTT CTGICTAACTA CTGICTAATGCTA CTTTGAATTG TCAAAAGGAT GAAACTTAAC GTTTACCCAG CGCGCCTGC CAAATGGGTC GCGGGGGACG		-	CAAATACTTG ATACTAACGC TATGATTGCG GTACATAATC	CCTANTANCT CGATTACTCA CGATCACCCACG	FACCATGTAC ATGGTACATG CGANGCAGA ACCACTANCT TGGTACATG TGGTACAGG CGANGCAGA TGGTACAGGGGGGGGGGGGGGGGGGGGGGGGGGG
CETTARCES C CETTARCES C CASTECROTS I CTASTECROS I TATTACCET I		CTCTCCGAGC GAGAGGCTCG ACCGTAAACC TCGCATTFGG CTACTGTCAG		COCAOCTOAN CCOCAOCTOAN CCOTCOACTT TATGCTATTT	TETCGACAT ACACGETETA TACAGTACTC ATCTCACGAG ACTAATTICAG TEGATTACAG TACTCAATTACAG TACTCA
• • •	GATTACATCA CTAATGTAGT TCTTCACCCG AGAMGTGGGC	ACTIANTAGANG TECTCTCTTG CTACCGCCTG GATGGCGGAC		AGCGGTGGGT ATGTTACATT TACAATGTAA GTATTACATT CATAGGAAA	TACHTETECH FIFTHTGAM TECTOCACTO CONTECTAT TECTOCACTO CONTECTAT ACGREGATE CONTECTANT ACGREGATE CONTECTANT GANTERATO TANACCCCC COTCATTACT TANACCCCCC COTCATTACT TANACCCCCC COTCATTACT TANACCCCCC COTCATTACT TANACCCCCC COTCATT
CCAMETTIC ACCUSTIANA GRATTGAAAC TRESCUSTITT GCCACAMINE ETTITACCICA: GGGGGGTEUN GLAVATRACC GGGGGGGTET GCCCTIANATC CCCTCTCGAA CGAGCATCGG	CCTANCCCTG GATTACATCA GGATTGGGAC CIVATATAGT AACACCACGG TETTCACCCG TTGCGGTGGC MIANGTGGGC	CTCACTCAGA TTTCACCACAC ACGTGGTGTG ACGTGGTGTG	CNTAATCCGG ATTCTCTGTC TAAGAGACAG AACGCTGGGG	STEGMANAT TIGGGACCCC STITANGGAG CCAGCCTGTA ANATTCCTC GGTCGGACAT CACAMANATA TITANCGTA	BEITION TACTITICCA CATA ATCANAGOT FIC TECTOCACTO AND ACGACTION AND ACGALOTION AND ACCATANATION AND ATTACOCOCC TAX ATTACOCOCOC TAX ATTACOCOCOC TAX ATTACOCOCOC
AGTTANATIA CACTOTCACO GTGACAGCO TTACCGCCA AATGGCGCCA	TTGCAACTOT AACGTTGACA CCATCCTGTA GGTAOGACAT	THOOCHCTOC CACCOCCGC GTGGCCGCG	THOODANTCC OUTHOUGHT ECACCCCAA	· .	
NCTATCCOOM ' TOATAGOCCT I ACACCTGOTC TOTTGOANCCAG GOCGTCCOGCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TCACTORDAT ACTGACACTA OCTCCTATCG CGAGGATAGC	CAACAGITIC GITGICAAAO ACGAGIGCGT TGCTCACGCA	MCAGGAGGT GAGGTTAGAN TTGTCCTCCA CTCGAATCTT TABIL TABIL TCAGGTTTCT CTAGAATCGG AGTCCAAAGA GATCTTAGCC		ACTICATION ACTICATATA TOCATATATA TOCATATATA ATALCTET
CCTCCCOOCC PORTECTED TO COOCCTOO TO COOCCTOO TO CCCOOCCTOO CCCOOCCTOO CCCOOCCTOO CCCOOCCTO CCCCOOCCTO CCCCOOCCCTO CCCCOOCCCTO CCCCOOCCCTO CCCCOOCCCTO CCCCOOCCCTO CCCCCOOCCCTO CCCCOOCCCCOOCCCTO CCCCCOOCCC	CCCTOTOTTC GGGACACAAG AFATACTGGG	CTOTCATTTA CACCOTAANT CCGGGAACOT GGCCCTTOCA	TIGICCICCA TIGICCICCA TCAGGITTET AGTCCANGA	ACOTOTANC Fort GOTACCACC CCATGATAGG	CCAGGOTMA CCAGATTER CAAATTER CACCCATT CACCCAGA CACCCAGA AAACTTACCA
27301 27401 27501	27601	27801	28001	28301	28501 28601 28701 28801

. 28901	COTAT TACABL	CTTCAACTCA	Car Therman	Attention	CHEACTERIES	CCAGCACCTIC	TCCCCATCACAT.	TREFFECTOR	CCAACTACAG	CCACCCACTC
t)))	COCCANCTIO			TACAGACICA				-		CICLP ROOM III
29001	TAACAGAGAT	GACCAACACA	ACCAACIBERS TOOTTOCOCC	CCGCCCCCTAC	CCCTGAATCT	TUTACCACAA AGATTT	ATACACCCCA TATGTCGCGGT .	ACTITICTUCE TCAAAGAEGG	TTTUTCAATA	ACTGGGATAA TGACCCTATT
29101	CTTCCCCATC	TOGTOGETICE ACCACCAAGA	CCATACKCCT	TATISTERSTA	TCGCANTATA	TTATCHEACT	CATCTRCTGE OT NOT NOT NOT NOT NOT NOT NOT NOT NOT	CTANANCICCA	AACOCACCCO	ACCACCCATC TOGTV ((7:TAG
29201	TATAGECCA	TCATTOTOCT	ACACCCAAAC	AATTATTAAA	TCTATAGATT	מבאכממארדמ	ANACACATGE	Territorer	TACAGTATGA	TTANATGAGA
	ATATCAGGGT	AGTAACACOA	DITTOGGITTO	TTACTACCIT	AGGTATCTAA	CCTGCCTGAC	THEFT	AGAAAAGAGA	ATCICATACT	AATTTACTET
	~ {	Thei								
29301	CATCATTCCT	CCAGTTTTTA			CTITITITION CONSCINCENC			GIFTETERACA	TCOAAGTAGA	CTCCATTC A
	. OTACTANOGA	GCTCNAMAT	ATAATGACTO	CCAACIACCC	GAANANCAC CICACGARTTR		TAACCGACGC	CAAAGAGTGT AGCTTCATCT	AGCINCATCT	GACOTAAO .T.
					Pg.I	į				
29401	OCCTICACAO						ACTIVITICATICA TODOCETITAT	TCCCCTTTAT		GACTOGGTT.T
	COGAROTOTC	ACATAMACGA		AATOCCTANA CAGTGGGAGT	GCTANTTACAC CITCYCIACTAG	GTCGGAGGTAG	TCACACCAGT AGCGGAAATA		OCTCACCTNA	CTGACCCATA
							Eco#!	_\$		
29501	OTOTOCOCTT	TOCATATOTO	AGACACCATC	CCCAGTACAG	GGACAGGACT	ATAGCTGAGC	THETHAGANT TETHTANTA		TOMATTHAC	TOTOACTTY "
	CACACGCGAA	ACCTATAGAG	TCTGTGTAGTAG	OCCITATION	CCTGTCCTGA	TATEGACTEG	ANGANTETTA AGAMATTANT		ACTITIVAARIO	ACACTGAAA.1
29601	CTGCTGATTA	THICKACCT	ATCTGCGTTT	TOTTCCCCOA	CCTCCAAGCC	TCAAAGACAT	ATATCATOCA	GATTCACTCG	TATATOGAAT	ATTCCAAGIT
	GACCACTAAT	AMCOTOGGA	TAGACCCANA	ACAAGGGGCT	GGAGGTYCUG	AGITTETGTA	TATAGTACGT	CTANGTGAGC	ATATACCTTA	TAACCITICAA
			•				Petl			
29701	OCTACAATGA	AAAAAGCGAT	CTITCCGAAG	CCFCECTTATA	TOCAMPCATO	TCTCTTATOG	TUTTETISCAG	TACCARCTER	GCCCTAGCTA	TATATCCCIA
	COATOTTACT	THITTCGCTA	GAAAGGCTTC	GOACCAATAT	ACGITAGTAG	AGACAATACC	ACAAGACGTC	ATCCTACAAT	COGRECOAT	ATATARKAT
29801	CCTTOACATT	GOCTOGMACO	CAATRGATGC	CATGAACCAC	CCANCITICC	محمدستحدم	TATOCTICCA	CTCCACAG	THOMPSECOS	COCCUTION
	COANCITUTA	CCGACCTTGC	GTTATCTACG	GIACTICGIO	CONTROVANCE	000000000	ATACGAAGGT	GACCITIONIC	AACAACOOCC GCCCBAAFA	GCCCANN' 1
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29901	CCAGCCANTC	Agenteacte	ACCTICACCC	ACCCCCACTO	AVATCARCTA		ACMININAMORNO.		CCCTADATCT	AGAAATTICAC
	OCTOBRENO	TOGGAGCGGG	TOGNACACIO	TROCKOTKIAC	TITAGICAL	CANATTACAT	TOTOCHOCHE	TACTGACTGT	GOGNICIAGA	TCTTTACCTO
10001	OCMATTATTA	CAGAGCAGCO	CCTGCTAGAA	AGACCCACAC	CACCOCCCGA	CCAACAGCGC	ATCAATCAAG	AGCTCCANGA	CATOUTTAAC	THECACCAGE
1	CCTTAATAAT	GRETEGREGE	GCACGATCTT	renocorece	GTCGCCGGCT	CGTTGTCGCG	TACTTAGETIC	TOGRAGOTTET	GTACCAATTO	AACTITICOTICA
30101	GCNAMOGGG	PATCETETOT	CICCIAMARC	AGGCCAAAGT	CACCTACGAC	AGTANTACCA	CCOGACACC	CCTTAGCTAC	ANGINECCAA	CCANGCOTI".
•	CONTINCOCC	ATAGAMACA	GAGCATITICS	PCCOOTTFCA	CTCCATCCTC	TCATIFATCE	accendade	GGAATCCATG	TTCAACGOTT	GCTTN:CV-AL:T
30201	GAAATTOOTO	GICATOOTOG	CAGNAAAGCC	CATTACCATA	ACTCAGCACT	CONTRACTOR	CGAAGGCTGC	AFFICACTICAC	CTTOTCAAGO	ACCTGARGAT
•	CTTTMCCAC	CAGTACCACC	CICITITICES	CENATICETAT	TGACTCCTCA	OCCARCITIE	GCTTCCGACG	TANGTONOTO	DAACAGTTCC	TOCACTOCTA
				Britt	1					
30301	CTCTGCACCC	TTATTWACAC	CCTOTACOOT	CTCHCCCCC TTATTANGAC CCTOTICGGGT CTCAAAGAT; TTATTCCCTT TAACTAATAA AAAAAAATAA	TTATTCCCTT	TACTATA		THANGCATCA	CTTACTTANA	ATCAGTTAGC
1	CACACCTICATO	AATAATICTG	AATAATTCTG GGACACGCCA	CAGITTCTAG	MTANGRIM	CAGITICIAS ANTANCEINA ATTICATIANT TITITITANT ATTICGIAGI	*********		GAATCAATTT	TACTCAATCC

• • • •			CCCCGAGGAA ACGTACATTY GAGCCTTGGG TITTGATTCA CTCGGGAACCC ANACTAAL ' FCCGTTTGAT GCTCANAACC AGGCAACTA CGAGTTTA' TACTTGTTA CAGCTTCAN	ANGANTAGE GECOAGETE GAGANGGE TGIANTIUS'S CTCTACCCG ACTTAACCA TCCTAACTA GOACTGATT AGGATTGAT CCTTAACCA	TCTCCTAACT CITCACTIVES AGAGGATTA CATCTGATT TTAAGGGGG TTTGGCTCCA AATTCCGTC AAACCGAGGT CCCAGAATAT TL. AAACTTAA GGGTCTTATA ACCTTGAAAT	CACOGRAMA CTECCAAAAG GTGCCATTIT GACOGRITIC
ACCOACOTT MATACCONCY TYCTOGRADA OCCITICASO	CTCTGGACGA GAGACCYGCT CCTCACAGTT GGAGTGTCAA	CACGACTCCA GTGCTGAGGT GTACCCTTAC CATGGGAATG ACTAAAGTAC	HOAFTTCATO AMOTIMOTO TITICAMIDAC AMOTIMOTIA TACAMIDACETT	OTTICCOOLA ATTAATOCAG TAATTACGTC AGOCTATOGT TCCGATACCA	ACCAGENCEA TOSTEGAGGT OTHTTORETG CAAAACCONC CCFTCCFGGA OUAAGGACCT	TCCAMATCT AGGTTTTTAGA
AGCTTCCTCC TYGAGGGGGG TGAGGGGGGG ACTTCTCGCGAT ATCTCCCAAT TAGGGGGTTA			CTTGCAAACT GAACGTTTGA CTTATACTTG GAATATGAAC	•	TETATACACA ACACCTITICA TACACTITICA ATCTCANGT CTANACATT	TATCAGCTTA
CRESTATIOC CACCATANCS TRUTTS ANA ANCANCETICT CTCCCTTICT GAGGGAAACA	CAAAATKAGG GTTTTACCCG AAGCTGGAAA TTAGACCTTT	CACAGOCCCC GTGTCCGGGG CCTCACCACC GGAGTGGTGG	ATATATATA ATATATATA ANACAGACG TITOTCTCCG	TTGACGCTAC AACTGCGATG CCTAGAATTT GGATCTFAAA	AAGCTAACTT TTCGATTGAA AAATACTTGC TTTATGAACG TTGAGTGCTA ACCTCACGAT	AFGCCFNACC TACGGATTOG
		TECATECHA TECATORICE CATCAGGCC GTAGTCCGGG GTAGTCCGGG	COCGENATA GRENCTATTA CACTGATANT TREATTCTCA AACTAAGAGT	CCCACCATC GRATCGGGG GRATTGATG CCCACCTACA TTYRECCATCG	MANTANTONT TETATTACTA TETACCETICA ACCCETICA AACTOCTTTT	AFACANACOC TESTOGATTT FATGITTECO ACAACCTAAA
PECTTGECCT CETTCEAGET AGGALGGGA FIATAFEGA CCGCACCCCC TATCTFCATG GREETTGATG ATAGAMGTW: AACTGATGCCT TTTCTTACTT TTGACACGGA AAAGAATGAG	TCCANTGGCA TGGTTGGCGA AGGTTACCGT AGGNGCGA AAAAACCAA GTGTATGTATA TTTTTTGGTT CAGTTTGTAT	CAACACACTC GTTGTGTGAG GCCCTGCAAA CGGGACGTT ACTTGAAAGA		TTTTATANA ANAANTATTT CACTGCCANG GTGACGGTTC ANAACAMAA	TAGGAAGAA ANCCTTGTT CTTAACAAAA GAATTGTTTT AFFAFAAGAF	
CHACHACACC 1 GECTOTICAT 6 NAGALAGETA C CCGOTICEC 1 GGCCAGGAGG 7	AGATEANTED CCACETETEN GOTTOGAGAGT	ACCAGCOCCC AGGAAACCTA TCCTTTCGAT	ACCCGTACC CCGTAGCAAC GCCATCGTTG TGTAGCAGGA ACATCGTCCT	CAGGGCCCTC GTCCCGGGAG TTAACCTAAG AATTGGATTC AAATCCCTC TTTAGGGGAG		OSCACANCET CCGTCTCAGA
CCAGTTATT CGECANATA CGECANATA CGECANATA CGECANATA CGECANATA CETATGCCTT	TATCCGAACC ATMOCTTOG CACTOTOMGC GTGACACTCG	GCACCTCTAA CGTGGAGATT CAGTGTCAGA GTCACAGAGTCT	ANCARCATOR ANCARCTETOR FECTORANCE FECTORANCE ACOFFICIANT	ANGACTAGGA TTCTGATCCT HAGII NAGCTTGAGG TTCGACCTCGAGGC GACCGAACAC TANGGTTTGAGG		GANATGGAGA TETTACTGAA CTTTACTCT AGAATGACTT
AMTHCTOT O THYMBACA G GAMTUTCAGT 1 CHTACAGTCA A GIATCCATAT C	ACAMACOCC AGAMACOCO AMANTOTANC TETTACATTG	RRSSE	ATTENTANCE ADACENCETA TESTECTORIAL CANCECTANA OFFICESTRAT	AACTAANTCT TTGATTTAGA CAATTCCAAA GITAAGGITT TCACCTAATG	ELSERE	CHITACCICT
30501 30501 30601	30701	31001	31201	31401 31501 31601	31701 31801 31901	32001

32101	TACATTOTC	AGTCAAOTTT	ACTTANACCS	ACACANANT	AAACCTCTCAA	CACTAMECAT	TACACTARAC	GGTACACAGG	NANCAGGINGA	CACAACTC
	ATTETARCAG	TCAGTTCAM	TOAATTIVEC	TURBLE	TTTX>:ACAIT	GICATIGGIA	ATGREATING	ccatetoree	THISTOCHET	GTGTTRACE :"
32201	AGTGCATACT TCACGTATGA	CTATOTCATT	TTCATCCCAG	TEXTURE TO A METER AND A METER	ACAM TACAT TOTTV:ATVITA	TATTACTTATA	THICK CACAT NACCATHUTA	CCTCTTACAC	TETETTCATAC AAAAAGTATG	ATTROCCON:
32301	AATAAAGAAT	COTTTOTOTT	ATCHTTCAC TACAMOTTIC	CACAMTANA	TTCAATITY'A AAGTPAAGGT	CTTFTAACT	NENGTANANA	CATTCAGTAG	TATAGECCEA	CCACCACATA
32401	GCTTATACAG		CTTAATCMA	CTCACACAAC	CCTAGTATTC	ANCETERCAC	CINCETECCA	ACACACAGAG	TACACAGTCC	בבני וניכני יי
277	CUMATATOTE		GAATARITE	GACTURETTE	GCATCATAAR	שואאאאנאמות	TANCKSACIATE	TOTOTOTOTO	ATGTGTCAGG	MARGAGO
10626	CGACCCGAAT	TITICOTAGE	TATCATCCCA ATACTACCCA	AACAGACATA TIGICIGIAT	TTCTTAGGTO ANIMATCCAC	TTATATTCCA ANTATAAGGT	CACCACATTICE OPSECAAAGE	TGTCGAGCCA ACAGCTCGGT	AACGCTCATC TTCCGAGTAG	AGTONTATE . TCACTATAF
32601	ATAMCTECE	COCCAGCIC	ACTIVACTIC	ATOTORCECTOT	CIT, N. A. WOLTER	AGCCACAGGC	TOCTOTCCAA	CTYCCUOTIG	CTTAACGGGC	GRETIANGTIA:
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32701	ANGTECACOC	CTACATOGGG	Gradaoteat	AATCGTGCAT	CAGGATAGEG		CONTROPICE GCAGCAGCUC	GCGNATANC	TOCTOCCOCC	OCCIACTOR
	TTCAGGTGCG	GATOTACCCC	CATCICAGTA	TTAGCACGTA	GPCCTATCCC		ACCACCACGA COTCOTOCO	COCTIAITIO	ACCIACOCCOG	COCCGAGGCA
	Pad									-
32801	CCTOCADOAA	TACAACATOO			ATTCGCACCO	CCCCX:NYCN?	ANTOCIACITY	GTCCTCCGGG	CACAGCAGCG	CACCCTOAT .
	GGACOTCCTT	AFOTTOTACC	GTCACCAGAG	GACTCCCTAC	TAAGCGTTAT	GCCCGTC07A	Trecococida	CAGGAGGCCC	GTOTCOTCOC	GTACCACT"
			Parl							
32901	TCACTTALAT	CAGCACACAGTA	ACTGCAGCAC	AGCACCACAA	TATTGETECAN	AATCCCACAG	TGCAAGGCGC	TOTATECAMA	GCTCATGGCG	OCCACCACAG
	AGTGAATTTA	Greenoreat	TOACOTCOTO	TECHGOTOTT	ATAACAAGTT	TTACCCTCTC	ACCITICORD	ACATAGOTTT	CONGRACCIC	CCCTOOTOTIC
33001	AACCCACOTO	GCCATCATAC	CACAAGCGCA	CCTACATTAA	GTORICANICC	CTCATABACA	CRCTRIGACAT	ARACATTACC	TCTTTTGGCA	TOTTGTAATT
	THOOOTIGEAC	U	GIGTTCGCGF	CCATCTAATT	CACCGCTOOG	GACTATITUT	CCGACCTIGTA	TPTOTAATOG	AGNAAACCOT	ACAACATTAA
	ē	Kpri								Pell
33101	CACCACCTCC	_		TAAACCTCTO ATTAAACATO	OCCICCATICCA	CCACCATCCT	MACCAGCTO	GCCAAAACCT	OCCCOCCOCC	TATACACTUS.
	GTOGTOGAGG	OCCATOOTAT	ATTOCACAC	TAATTTGTAC	CGCGGTAGGT	CONTOCTACCA	TTOXITOGAC	COCTITICGA	cooccecca	ATATOTORO:
	E							Eranv		
33201	ACCOUNTCOOL	GACTOGAACA		ACAGCCCAGG	ACTOSTANCO		ATCCTCGTCA	TCATATCARE	GTTOGCACAA	CACAGOCACA
	recerronce	CTGACCTTOT	TACTOTORCACC	acticisatics	TONOCATIOS	TACCTAGING	TACCAGCAGE	ACTATAGETTA	CAACCGROTT	GTGTT:CGTGT
	•									15.1
33301	COTOCATACA	CTTCCTCA00	ATTACARGCT		TACANCCATA	TCCCAGGGAA	CARCCATTC		GTAMATCCCA	CACTRICAGRE
	OCACOTATOT	GANGGAGTCC	TAATGTTCGA	GGAROCCCCA	ATCTINGTAL	AGGGTCCCTT	CITICOCITANG	GACTTAGTEG	CATTITAGGGF	GTOACCICE
33401	MONCETCOC	ACCITANCICA	COTTOTOCAT	TOTICANGING	TANCAPPECOS	GENERALING	ATGATCCTCC		CCCCCCTTC	TCTCAM
	TTCTOGACCO	TOCATTOAGE	OCANCALIGTA	ACASTETICAC	ANTOFAGEC	נייזכיזכיזכ	TACTACASACIO	TCATACCATC	OCCCCAANG	ACAGAGETET
33501	CONCOTACAC	GATCCCTACT	GINCOUNTED	CTICCOMINCA	ACCCACATCG	TOTAL COLLEGE			OCCOGACOTA	CTCATATTE.
	CCTCCATICTO	CTAGGGATGA	CATOCCTCAC	GCGGCTCTGT	TOCCTCTAGE	ACMACCARCA	TCACAGTACG	GTTTACCTTO	COCCTCCAT	CACTATAAN

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Figure 15V

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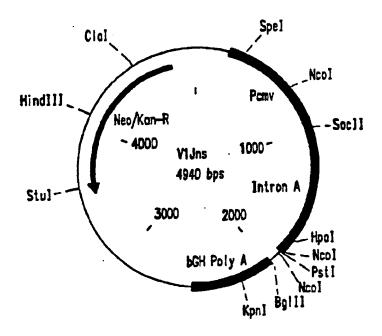
35301	CATITITANDA	AAACTACAAT	TECENACACA	TACAAGTTAC	Trencetal		ACCEGECEEG	TTCCCACGCC	CCCCCCACG	TENCAMETE
	GTAMATHCT	TTTGATGTTA	ACCURICACT	ATCITICAATG AGGCGGGATT	MACCHARIATT	THOGATOCAG	Transcensor.	AGGGTGCG	Occocoatac	ACTICITATIONS
•						Pa	on many			٠
15401	CALTERIOR	TTARCATATE	Carrier Barr	TO CASTO CONTRACTOR	*ATATESTO	Archivers	TE A POST	Carameters		
	GTOGGGGAGT	AATAGTATAA	CCGANGTTAG	GTTTIATTCC	ATATAATAR	TACTACAATT	AATTCTTAAG	CCTMGACCCT	OCCUPACE	CTACCCOANG
35501	CCCAFFATOA	TICTICIOS	MCCOCCOC	ATCCCCATUC	CCCCCTTCCA	COCCATOCTO	TECNOGENOG	TAGATCIACGA	CCATCAGGGA	CACCTICANO
	GCOTAATACT	ANGANGAGCO	AAGGCCGCCG	TAGCCCTACG	GOCGY: NATURE	CCCXCTACGAC	AGGTCCGTCC	ATCTACTOCT	OCTAGRECET	GICGAAGTIC
35601	GCCAGCAAAA	GOCCADONAC	COTTABANAGO	CCCCUTTGCT	OCCUPATION	CATAGACTCC	GCCCCCCTGA	CONGCATCAC	ANMATCOAC	OCTCAAGTC/.
	COORCOTTIT	CCCCTCCTTG	GCATTITICE	GGCGCNACGA	CCCCANNG	GTATCCCAGG	COCCOCCACT	OCTOGTAGTO	TTTTAGCTG	CCACTTCACT
35701	CACOTOCCAA	ACCCOACAG	GACTATAAAG	NTACCAGACO	THECECETA	CANCETICET	CONOCOUNT	ccrorrecea	CCCTGCCGCF	TACCCOATAC
	CTCCACCGCT	THOCOCHOTC	CICATATTIC	TATOGRECOC	AAAGTGGGAC	CTTCGAGGGA	CCACCCCAGA	GGACAAGGCT	COTACOCCOA	ATOCCCTATY:
35801	CTOTOCOCCT	TICHCCCTTC	GOGARGCHTO	GCGCTTTCTC	ATARCTCACG	CTUTARGETAT	CTCANTICOO	TOTAGGTEGT	rectecaso	CTOOCITIES.
	GACAGGCGGA	ANGNODANG	CCCTTCGCAC	COCCEANAGAG	TATEGRAPHOE	GACATCCATA	CANTICANCIC	ACATCCAGCA	AGCGAGGITIC	GACCCGACA!
35901	TOCACOMOC	CCCCOPICAD	CCCGACCGCT	OCCCCTTATC	COGTANCTAT	CONCINGAGE	CCANCCCOST	ANGACACGAC	TTATCOCCAC	TOOCAGCAGY.
	ACCITOCITION	COOCCAAGTC	COGCIECCO	COCOGNATAG	OCCAPTIGATA	GCAGAACTCA	GGTTGGGCCA	THETOTOCHO	AATAGCGGTO	ACCONCORC
36001	CACTOOTAAC	AGGATTAGCA	GACCGACCTA	TOTAGGCGGT	CACTACACACT	TETTOANGTO	GTOCCTANC	TACOCCTACA	CTAGAAGGAC	ACTATITION
	OTOACCATTO	TECTAATEGE	CTCGCTCCAT	ACATCCGCCA	CGATGITCTCA	AGNACTITCAC	CACCOGATTO	ATOCCOATOR	DATICTICCTO	TCATAAACCA
36101	ATCTOCOCTC	TOCTOARGCC	AUTTACCTTC	CCANANACAC	THASTAGETE	TTGATCCGGC	ANACAAACCA	CCCCTGGTAG	COCHOCHETT	trigringe.
	TADACOCORO	ACCACTTCGG	<i>fcMTGGMG</i>	CCTTITICE	NACCATCGAG	AACTAGGCCG	THOTTOGE	GOCCOACCATIC	OCCYCCANA	AMCMARCET
36201	ACCACCACAT	7	AAANANGGAT	CTCAAGAAGA	TECTFIGATE	TITICINCO	OCTETE ACOC	TCAGTOGNAC	DANACTCAC	GTTANGGEN
	fcorcorct	ATOCOCOTCT	TTTTTTCCTA	GAGITCTICT	ACCIMANCTAG	ANMGATTACC	CCAGACTIOCO	AGREACETTO	CTTTTGAGTO	CAATICCCT
36301	THOOTCATO	AGATTATCAA	AAAGGATCTT	CACCTAGATC	CTTTTAAATC	ANTCTANNOT	ATATATOAGT	AAACTTGGTC	TGACAGTTAC	CATCCTTA
	AACCAGTAC	TCTANTAGET	TTTCCTAGA	GTOGATICTAG	GAMMATITTAG	TTAGATTTCA	TATATACTCA	THEMOCAO	ACTOTCAATO	CTINCONATIO
36401	TCAGTGAGGC	_	OCCUPATION	TATTACGITE	ATCCATAGET	OCCTOACTCC	CCCTCCTCTA	GATAACTACG	ATACOGGAGG	OCTTACCATY:
	AGICACICCO	TOGATAGAGE	COCTAGACAG	NTWARGENAG	TACCTATCAA	CREACTENED	OGCAGCACAT	CTAINCAROC	TATOCCCTCC	CKWATKKITAU
36501	TOOCCCCAOT	GCTOCANTOA	TACCCCCAGA	CCCACGCTCA	CCCCCTCCAG	ATTTATCAGE	AATAAACCAG	CCACACOCOAA	GGGCCGAGCG	CACIMICAGOT
	ACCOGGGTCA	COACOTTACT	ATOCCOCTCT	GOOTGCGAGT	GCCCGAGGTC	TAANTAGTCO	TATTIGGIC	GOTCOCCT!	CCCCOCLCCC	CHCTHCACCA
36601	CCTGCARCTT	TATCCOCCTC	CATCCAOTCT	ATTAATTCIT	GCCCAGGNATC	TATASTANGE	AGITCGCCAG	TTAATACTTT	OCCCAACGTT	GITTCACCATTG
	OGACOTTGAA	APACCCCCO	GTAGGTCAGA	TAATTAACAA	COGCCCTTCG	ATCTCATTCA	TCANDCIACTC	ANTTATCANA	COCOTTOCAA	CANCOCTAAC
36701	CTACAGGCAT	COTOCHOICA	COCTOSTOST	Trooppredic	TTCATTCAGC	Techaritee	AACCATCAAG	GCCAOTTACA	TCATCCCCCA	TOTTOTACAA
	GATOTCCOTA	GCACCACAGT	OCCAOCACTA	AACCATACCG	AAGTAAGTCO	ACICCANGCG	TICCTACTIC	COCTICAATOT	ACTAGGGGGGT	ACAACACGTT
			75	***						
36801	ANAGOOGIT	ACCICCITICO	GECCECCOAF	COTTOTACAGA	ACTAACTING	CCGCAGTGTT	ATCACTCATG	OTTATOCCAG	CACTOCATAA	TTCTCTTACT
	TTTCCCCAA	TCCAGGAAGC	CAGGAGGCTA	GCANCAUTICT	TCATTCAACC	COCCICCACAA	TACTICAGIAC	CANTACCOTC	OTGACGTATT	ANGAGAATGA
36901	GTCATGCCAT	CCGTAAGATG	certicion	ACTOGREGACT	ACTUANCOA	GICATICTOA	CAATACTCTA	TOURCEACE	GAGTIGGETET	TOCCCORCUT
	CAGTACGGTA	OCCATICIAC	GAMAGACAC	TOACCACTCA	TCAGTTCGTT	CARTANGACT	CTTATCACAT	ACCCCCCTCC	CTCAACGAGA	ACCICCCCCA

figure 15W

PMRKAdSgag MER682

CHACACGODA FANTACCOCO CCACATARCA GINCTITIANA AGITETENTE ATTRAMANC GITCTHEGGS GEGANIACTE TEMBGRATET TREGGRATET TRACCATARCA ATTRACANA GITTHEGGS AGITCCTAGA ATTRACANA GITTHEGGS AGITCCTAGA AGICCANANT CTTTTMETT CACAMITEMA GITCHEGGS ANGECANANT CTTTTMETT CACAMITEMA GITCHEGGS ANGECANANT CCCATAGAT TOTACATA AGICCACTE GITTHTGTT CACAMITEMA GITCHEGGS ANGECTAGA AGICCACACG GITTHTGTT AGAMITEMA GITCHEGGS ANGECTAGA AGICCACACG ATTRACACA ATTACACACA ATTACACACA ATTACACACA AGAMINET ANGACANANT AGAMITEMA GITTHTANTA CTCCATACACA AGTCCACACA ACCOCACACA ACCOCACACA AGAMINETT TOCCANANT AGAMITEMA ACCOCANANT ACCOCAMANTA AGAMITEMA ACCOCANANTA ATTACACANANTA AGAMITEMA ACCOCANANTA AGAMITEMA ACCOCANANTA AGAMITEMA ACCOCANANTA ACCOCANANTA AGAMITEMA ACCOCANANTA AGAMITEMA ACCOCANANTA AGAMITEMA ACCOCANANTA ACCOCANAN	
CCTC TCANGGATC COAG AGTTCCTAG COAG CAAAACAC CCTC GTTTTTTGTC CTTA TCAGGGTTT AAT AGTCCCAA COAC GTCTAAGA CCTG CAGATTCT CAC CAGATTCT CAC CAGATTCT	CATGACATTA ACCTATAMA ATARGEGIAT CACCAGGEE TTICGTETTE ANGANTISSA TEGRATET TANT (SEQ ID NO: 27) GTACTGRAAT TEGRATETT TATECECATA GTECTCCAGG AMGEARANG TTETTAMECT AGGETAMA ATTA (SEQ ID NO: 28)
G GCGAAAA C C CCCTTTTT T TCTCACT A AGACCCC T GAAGCCC A CTTCOTT TI GCCCCC A COCTGG	TAAT TE
CCANGANGICE CACTANGICE CLASTACTE CLASTANTAN GTTATANT GGGCTTTTT	BamHi GA TY CGAATTA CT AGGCTTAM
TANCETTTO CTTTTM:TTT GANATIANA CTTCLTTTT GANZIANAA CTTACTTTC GCGTGTANIG	Bar AAGAATITGGA TTCTTAAGCT
TCACTACTORY TCACTACTACT TCTTICATAT TCTTICATAT AGAMETRITA TACTTCATATATA TACTTCATATATA TACTCATATATA AGGRESSTOTT	Treaterte
GAACTTTAAA CTTGAACTGA ACTCAACTGACT TAXXCTTGACT AAAACAAATT	CACGAGGCC
CCACATARCA GCTGTATCGT CCACTCGTGC GCTGACACGG GCCGCTGTGCGG ATTTAGAAAA TAAATCTTTT	ATACGCGTAT TATCCOCATA
TANTACCUCU ATTATOGCOC ACCTACATTO AGCTACATTO ATTTOGATOT TANCTTACA	ACCTATAMA TOXATATITI
CHACACAGA GTTUTECCTT GAGANTCCAGT CTCTAGOTCA GCCGCANAN CCGCGATACAT CGCCTATOTA	
37101 37101 37201 37301	37401

Figure 15X



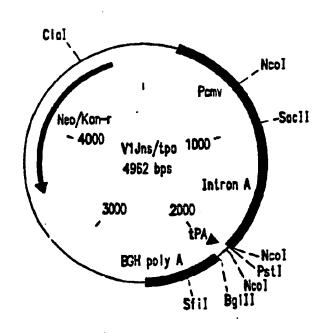


FIGURE 16

AGATCTAD	CATGGCCCCCATCTCCCCCATTGAGACTGTCCCTG	GAAGCTGAAGCCTGGCATGGATGGCCCCAAGGTGAA
Bg/11	MetAloProlieSerProlleGluThrVolProVi	ilysleulysProGiyMetAspGiyProLysVolly
•	1 10	20

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGAGGGCAAAATCTCCA sGInTrpProLeuThrGluGlu_yslieLysAlaLeuVolGluIleCysThrGluMeLGluLysGluGlyLysIleSerL 30 40 50

AGATTGGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGAACGACTCCACCAAGTGGAGGAACCTGGTG
ysleGlyProGluAsnProTyrAsnThrProVolPheAlolleLysLysLysLysAspSerThrLysTrpArgLysLeuVol
50 70

GACTICAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCTGGCCTGAAGAA AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluVolGlnLeuGlylleProHisProAloGlyLeuLysLy 80 90 100

GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGGATGCCTACTTCTCTGTGCCCCTGGATGAGGACTTCAGGAAGTACACTG slyslysSerVolThrVolLeu<u>Alo</u>VolGlyAspAloTyrPheSerVolProLeuAspGluAspPheArgLysTyrThrA 110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC loPheTnrlieProSerlieAsnAsnGluThrProGlylleArgTyrGinTyrAsnVoiLeuProGlnGlyTrpLysGly 140 150

TCCCCTGCCATCTTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA SerProAloliePheGinSerSerMetThrLysileLeuGluProPheArgLysGinAsnProAsplleVollleTyrG1 160 170 180

TGCTGAGGTGGGGCCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC euleuArgTrpGTyleuThrThrProAsplysLysHisGInLysGIuProProPheleuTrpMetGTyTyrGTuLeuHis 220 230

CCCGACAAGTGGACTGTGCACCCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG ProAsplysTrpThrVoIGinProIieVoileuProGiulysAspSerTrpThrVoIAsnAspIieGinLysLeuVoIGI 240 250 260

CAAGCTGAACTGGGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCCC yLysleuAsnTrpAloSerGinlleTyrProGlyileLysVolArgGinleuCysLysleuLeuArgGlyThrLysAloL 270 280 290

FIGURE 17A

GCCCTGTACTATGACCCCTCCAACGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA GlyVoiTyrTyrAspProSerLysAspLeulieAloGiulieGinLysGinGlyGinGlyGinTrpThrTyrGinlieTy 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGGCCCCACACCAATGATGTGAAGCAGCTGA rGInGIuProPhelysAsnleulysThrGIyLysTyrAIoArgMelArgGIyAIoHisThrAsnAspVoilysGInLeuT 350 350 370

CTGAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGinLyslieThrThrGluSerlieVollleTrpGlyLysThrProLysPheLysLeuProlleGinLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATTGTGGGGGCTGAGACCTTCTATGTGGCTGGGGGCTGCCAACAGGG uVoiLysLeuTrpTyrGInLeuGluLysGIuProileVolGlyAloGluThrPheTyrVolAloGlyAloAloAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC LysThr<u>Alo</u>LeuGInAlolleTyrLeuAloLeuGInAspSerGlyLeuGluVolAsnIleVolThr<u>Alo</u>SerGInTyrAlo 480 490 500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG cleuGiyllelleGinAicGinProAspGinSerGiuSerGiuLeuVolAsnGinIlelleGiuGinLeuIleLysLysC 510 520 530

ATCAGGAACGTGCTGTTCCTGGATGGCATTGACAACCCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT
11eArgLysValleuPheleuAspG1y11eAspLysA1oG1nAspG1uHisG1uLysTyrHisSerAsnTrpArgA1oMe
560 570 580

FIGURE 17B

CGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCCTAAGGAGATTGTGGCCTCCTGTGACAAGTGCCAGCTGAAGGGGGAGG tAlaSerAspPheAsnLeuProProVolVolAlaLysGluIleVolAlaSerCysAspLysCysGlnLeuLysGlyGluA 590 600 610

GCTGTGCATGTGGCCTCCCGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT AlovalHisValAloSerGlyTyrlleGluAloGluVollleProAloGluThrGlyClnGluThrAloTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGCTG uLysleuAloGlyArgTrpProVolLysThrlieHisThrAloAsnGlySerAsnPheThrGlyAloThrVolArgAloA 670 680 690

CCTGCTGGTGGGCTGGCATCAACCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGGTGGTGGCCTCCATGAAC IoCysTrpTrpAloGlylleLysGInGluPheGlylleProTyrAsnProGInSerGInGlyVolVolAloSerMetAsn 700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT LysGluLeuLysLyslielleGlyGlnVolArgAspGlnAloGluHisLeuLysThrAloVolGlnMetAloVolPhell 720 730 740

CCACAACTTCAAGAGGAAGGGGGCATCGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPhelysArglysGtyCtylleGtyGtyTyrSerAloGtyGtuArglteVolAsplieIteAloThrAsplieG
750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG InThrLysGIuLeuGInLysGIn!ieThrLysI!eGInAsnPheArgVoITyrTyrArgAspSerArgAsnProLeuTrp 780 790

AAGGCCCTGCCAAGCTGCTGTGGAAGGCCGAGGGGGCTGTGGTGATCCAGGACAACTCTGACATCAAGGTGCTGCCCAG LysGlyProAloLysLeuLeuTrpLysGlyGluGlyAloVolVollieGlnAspAsnSerAsplleLysVolVolProAr 800 810 820

AAAGCCCGGGCAGATCT (SEQ ID NO: 3) χ_X (SEQ ID NO: 4)

FIGURE 17C

(within SEO 10 NO: 7) (within SEO 10 NO: 8) RoSerGiulieSerAidProlieSerProlieGiuThrVoiProVoilysLeutysProGlyMetAspGly 10 20 20

FIGURE 18

```
- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT
                                                           -42
WT
        - ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC
OPT
           M G G K W S K R S V P G W S
                                                           -14
        - ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT
WT
        - ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC
DPT
           TVRERMRRAEPAAD
                                                           -28
        - AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA
                                                           -126
WT
        - AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC
DPT
           RVRRTEPAAVGVGA
                                                           -42
        - GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC
                                                           -168
WT
         . GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC
OPT
            V S R D L E K H G A 1 T S
                                                            -56
         - AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA
                                                            -210
WT
         - AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC
OPT
            NTAATNADCAWLEA
                                                            -70.
         - CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA
                                                            -252
WT .
             - CÁG GÁG GÁC GÁG GÁG GTG GGC TTC CCC GTG ÁGG CCC CÁG GTG
OPT
            DEDEEVGFPVRPQV
                                                            ·84
         - CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC
                                                            -294
 MI
         - CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC P L R P M T Y K G A V D L S
 OPT
                                                            -98
         - CAC TIT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC
                                                            •336
 WT
         - CAC TIC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC
 OPT
            HFLKEKGGLEGLIH
                                                            -112
         - TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC
                                                             -378
 WT
         - TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC
 OPT
          SQKRQDILDLWVYH
                                                             -126
         - ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG
                                                             -420
 WT
          - ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC
 OPT
             TOGYFPDWDNYTPG
                                                             -140
```

FIGURE 19A

```
. CCA GGA ATC AGA TIT CCA TTG ACC TTT GGA TGG TGC TTC AAG
                                                      -462
 WT
         - CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG
 OPT
           PGIRFPLTFGWCFK
                                                       -154
        - CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA
                                                       -504
 WT
         - CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG
 OPT
           LVPVEPEKVEEANE
                                                       -168
        - GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG
                                                       -546
 WI
        - GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC
. OPT
           G E N N C L L H P M S Q H G
                                                       -182
        - ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC
                                                       -588
 WT
          11 111 111 11 11 111 111 111 111 111 111 111 111 111
         - ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC
 OPT
           I E D P E K E V L E W R F D
                                                       -196
         - AGC AAG CTA GCA TIT CAT CAC GTG GCC CGA GAG CTG CAT CCG
                                                       -630
 WT
          - TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC
 OPT
           SKLAFHHVARELHP
                                                       -210
                                                       -651
         - GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30)
 WT
          111 111 111 111 111 111 1
         - GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9)
 OPT
                                                       -216
           E Y Y K D C (SEQ ID NO: 10)
```

FIGURE 19B

CATBOSTCTTTCTGGGGCCCCTCCTTGGGTCTGCCACC ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC . . . VIJns/nef

Srf1 Bg111

. . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGAGAICIGCCCTTCTAGTTGCCAGC (SEQ 1D NU: 38)

H P E Y Y K D C * (contained within SEQ 1D NO: 10:

V1Jns/nef(G2A.LLAA)

Srff Balli
CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGGAGAICIGCTGTGCCTTCTAGTTGCCAGC (SEQ TD NO: 39)
H P E Y Y K D C * (contained within SEQ ID NO:14)

VlJns/tpanef & VlJns/tpanef(LLAA)

PSt CATBGGTCTTTTCTGCAGTCCTTATATCTAGATCACC ATG GAT GCA ATG ANG AGA GGG CTC TGC TGT GTG

CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG AIC TCC AAG AGG TCC GTG CCC ...

Srf1 Bg111

Srf1 Bg111

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGAGAICIGCTGTGCCTTCTAGTTGCCAGC (SEQ 1D NO: 40)

H P ; E Y Y K D C * (contained withon seq ID NO: 16)

FIGURE 20

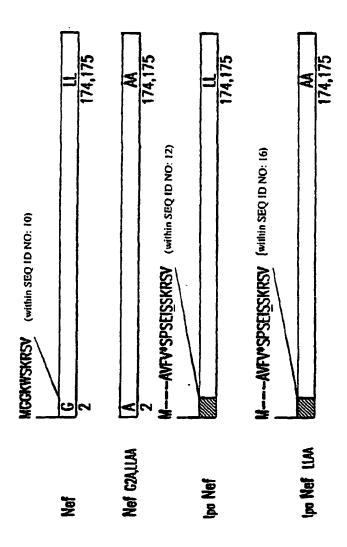


FIGURE 21

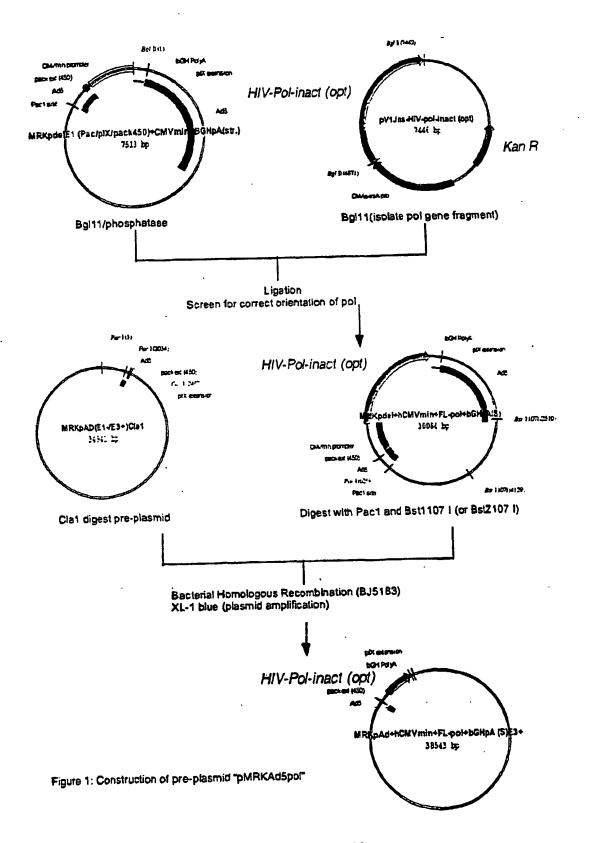


FIGURE 22

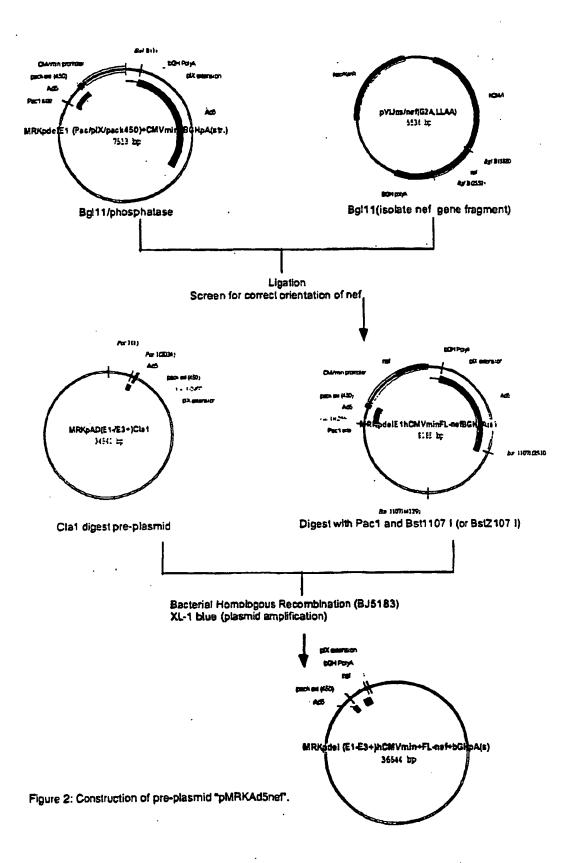
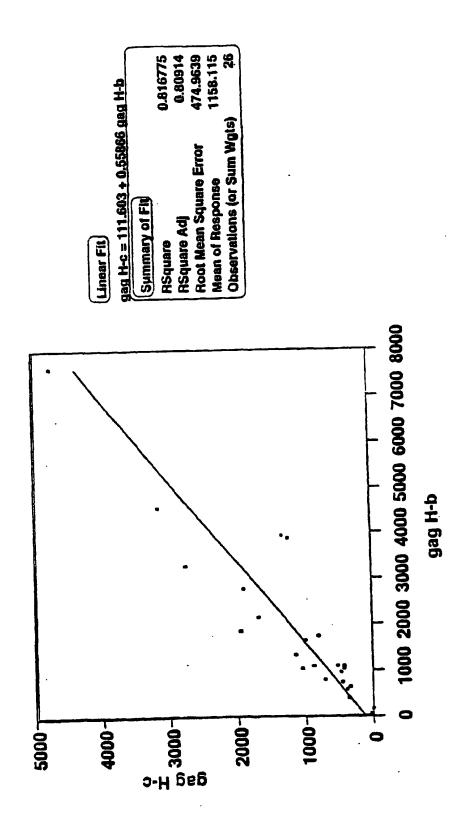
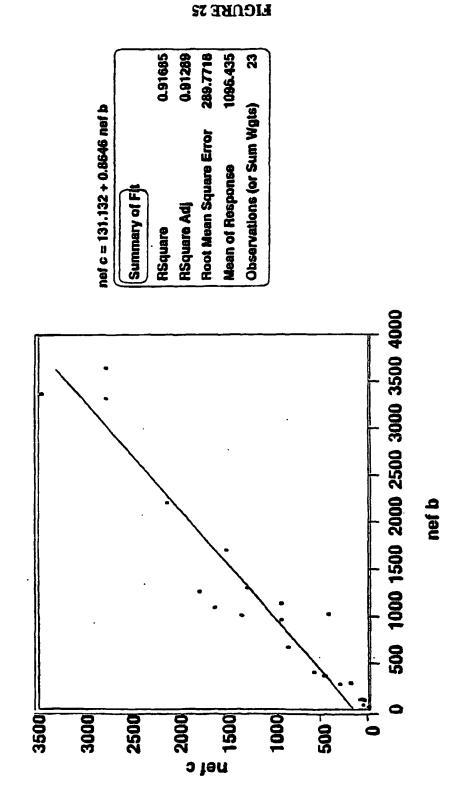


FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



MRKAd5pol MER1062 (MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

1	CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
_	GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC
51	GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
	CCCCACCTCA AACACTGCAC CGCGCCCGC ACCCTTGCCC CGCCCACTGC
101	TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
TOT	ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT
1 5 1	GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
151	CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC
	GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
201	CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC
	CITCACIGIT ANAMOUNCE COLUMN
	CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA
251	GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT
	GCATTGGCTC ATTCTARAGE CONTENTS
	AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
301	TCACTITAGA CITATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT
	TCACTITAGA CITATIAAAR CACAATGGG
	GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
351	CCCGGCGCCC CTGAAACTGC CAAATGCACC TCTGAGCGGG TCCACAAAAA
	CCCGGCGCCC CYGAAACIGG CAARIOGAGC LOTS
	CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
401	CTCAGGTGTT TTCCGCGTTC CGGGCCTTTC AACCGCAAAA TAATAATATC
	GAGTCCACAA AAGGCGLAAG GCCCAGIIIC ISIGGGGG
	GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
451	CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA
	CGCCGGCGCT AGGIAACGIA IGCAACAIAG GIAING
	TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
501	ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG
	ATATAACCGA GTACAGGTTG TAATGGCGGT ACADGCGGT
	TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
551	ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT
	ATCANTANT ATCATTAGTT ARTOCCCCAG TATTAGTT
	TEGASTTCCG CETTACATAA CTTACESTAA ATEGCCCCCC TEGCTGACCC
601	TEGRETTCCE CETTACATAR CTTACGGTAR RESCUES ACCEACTEGC ACCTCARGEC GCARTETATT GARTGCCATT TACCGGGCCG ACCGACTGGC
	ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGGG TO
	CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
651	GCCAACGACC CCCGCCCATT GACGICAATA ATGACCATAC AAGGGTATCA
	GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGGTTAT
	AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
701	AACGCCAATA GGGACTTTCC ATTGACGICA AIGGGTACTC ATAAATGCCA TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA
	TTCCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCAGGT
	CARRELA COMON ANCANATORE AAGTACGCCC
751	AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
	TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG
801	CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
	GGATAACTGC ACTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT
853	CATGACCTTA TEGGACTITC CTACTTEGCA GTACATCTAC GTATTAGTCA
	CATGACCTTA TOGGACTATA ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

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901				AGTACATCAA TCATGTAGTT	TGGGCGTGGA ACCCGCACCT
951				CTCCACCCA GAGGTGGGGT	
1001				GGACTTTCCA CCTGAAAGGT	AAATGTCGTA TTTACAGCAT
1051	ACAACTCCGC	CCCATTGACG	CAAATGGGCG	GTAGGCGTGT CATCCGCACA	ACGGTGGGAG
1101	GTCTATATAA	GCAGAGCTCG	TTTAGTGAAC	CGTCAGATCG GCAGTCTAGC	CCTGGAGACG
1151	CCATCCACGC	TGTTTTGACC	TCCATAGAAG	ACACCGGGAC	CGATCCAGCC
1201	TCCGCGGCCG	GGAACGGTGC	ATTGGAACGC	TGTGGCCCTG GGATTCCCCG	TGCCAAGAGT
1251				CCTAAGGGGC TGAGACTGTG	
1301		·		ACTCTGACAC AGCAGTGGCC	
	ACTTCGGACC	GTACCTACCG	GGGTTCCACT	TCGTCACCGG	GGACTGACTC
1351				ACTGAGATGG TGACTCTACC	
1401	-			CTACAACACC GATGTTGTGG	
1451				GGAAGCTGGT CCTTCGACCA	
1501				GAGGTGCAGC CTCCACGTCG	
1551		• • • • •		TGTGACTGTG ACACTGACAC	
1601				AGGACTTCAG TCCTGAAGTC	
1651				ACCCCTGGCA TGGGGACCGT	
1701				CTCCCCTGCC GAGGGGACGG	
				GGAAGCAGAA CCTTCGTCTT	
1801				GTGGGCTCTG CACCCGAGAC	
				•	



	CCCCCTGAC	CACCCCTGAC	AAGAAGCACC	AGAAGGAGCC	CCCCTTCCTG
1901	CCCCGGACTG	GTGGGGACTG	TTCTTCGTGG	TCTTCCTCGG	GGGGAAGGAC
1951	TGGATGGGCT	ATGAGCTGCA	CCCCGACAAG	TGGACTGTGC	AGCCCATTGT
•••	ACCTACCCGA	TACTCGACGT	GGGGCTGTTC	ACCTGACACG	TCGGGTAACA
2001	GCTGCCTGAG	AAGGACTCCT	GGACTGTGAA	TGACATCCAG	AAGCTGGTGG
		TTCCTGAGGA			
2051	GCAAGCTGAA	CTGGGCCTCC	CAAATCTACC	CTGGCATCAA	CCACTCCGTC
		GACCCGGAGG			
2101	CTGTGCAAGC	TGCTGAGGGG	CACCAAGGCC	CTGACTGAGG	ACTAGGGGGA
		ACGACTCCCC			
2151	GACTGAGGAG	GCTGAGCTGG CGACTCGACC	AGCTGGCTGA	CTTCTCCCTC	TAGGACTTCC
2201	AGCCTGTGCA	TGGGGTGTAC	TATGACCCCT	CCAAGGACCT	GATTGCTGAG
227-	TCGGACACGT	ACCCCACATG	ATACTGGGGA	GGTTCCTGGA	CTAACGACTC
2251	ATCCAGAAGC	AGGGCCAGGG	CCAGTGGACC	TACCAAATCT	ACCAGGAGCC
	TAGGTCTTCG	TCCCGGTCCC	GGTCACCTGG	ATGGTTTAGA	TGGTCCTCGG
2301	CTTCAAGAAC	CTGAAGACTG	GCAAGTATGC	CAGGATGAGG	GGGGCCCACA
		GACTTCTGAC			
2351	CCAATGATGT	GAAGCAGCTG	ACTGAGGCTG	TGCAGAAGAT	CACCACIGAG
		CTTCGTCGAC			
2401	TCCATTGTGA	TCTGGGGCAA	GACCCCCAAG	TTCAAGCTGC	CCATCCAGAA
					GGTAGGTCTT
2451	GGAGACCTGG	GAGACCTGGT	CCTCACTCACIA	GACCGTCCGG	ACCTGGATCC TGGACCTAGG
2501	CTGAGTGGGA	GTTTGTGAAC	ACCCCCCCC	TGGTGAAGCT	GTGGTACCAG
	GACTCACCCT	CAAACACTTG	TGGGGGGGG	ACCACTTCGA	CACCATGGTC
2551	000030330	· ACCCCATTGT	GGGGGCTGAG	ACCTTCTATO	TGGCTGGGGC
2551	CTGGAGAAGC	TCGGGTAACA	CCCCGACT	TGGAAGATAC	ACCGACCCCG
2601	TGCCAACAG	GAGACCAAGO	TGGGCAAGG	TGGCTATGT	ACCAACAGGG TGGTTGTCCC
				•	
2651	GCAGGCAGA	GGTGGTGAC	CTGACTGAC	A CCACCAACC	GAAGACTGCC
	CGTCCGTCT	r ccaccactgo	GACTGACTG	r ggragriag.	CITCIGACOO
2701	CTCCAGGCC	A TCTACCTGG	CCTCCAGGA	C TCTGGCCTG	G AGGTGAACAT
	GAGGTCCGG	T AGATGGACC	GGAGGTCCT	G AGACCGGAC	C TCCACTTGTA
		c	CCCTGGGCA	T CATCCAGGC	C CAGCCTGATC
2751	TGTGACTGC	G AGGGTCATA	C GGGACCCGT	A GTAGGTCCG	G GTCGGACTAG
	VCVC 10VCQ				

Figure 26 C

2851	GAGAAGGTGT CTCTTCCACA			CACAAGGGCA GTGTTCCCGT	
2901				CATCAGGAAG GTAGTCCTTC	
2951				ATGAGAAGTA TACTCTTCAT	
3001	TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCTGTGG	TGGCTAAGGA
	ACCTCCCGAT	ACCGGAGACT	GAAGTTGGAC	GGGGGACACC	ACCGATTCCT
3051				GAAGGGGGAG CTTCCCCCTC	
3101	GGCAGGTGGA	CTGCTCCCCT	GGCATCTGGC	AGCTGGCCTG	CACCCACCTG
	CCGTCCACCT	GACGAGGGGA	CCGTAGACCG	TCGACCGGAC	GTGGGTGGAC
3151				GTGGCCTCCG CACCGGAGGC	
3201				GGAGACTGCC CCTCTGACGG	
3251	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
	ACTTCGACCG	ACCGTCCACC	GGACACTTCT	GGTAGGTGTG	ACGGTTACCG
3301				GCCTGCTGGT CGGACGACCA	
3351				CCAGTCCCAG GGTCAGGGTC	
3401	CCTCCATGAA	CAAGGAGCTG	AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG
	GGAGGTACTT	GTTCCTCGAC	TTCTTCTAGT	AACCCGTCCA	CTCCCTGGTC
3451	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG	GCTGTGTTCA	TCCACAACTT
	CGACTCGTGG	ACTTCTGTCG	ACACGTCTAC	CGACACAAGT	AGGTGTTGAA
3501	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG	AGGATTGTGG
	GTTCTCCTTC	CCCCCGTAGC	CCCCGATGAG	GCGACCCCTC	TCCTAACACC
3551	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
	TGTAGTAACG	GTGTCTGTAG	GTCTGGTTCC	TCGAGGTCTT	CGTCTAGTGG
3601	AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG
	TTCTAGGTCT	TGAAGTCCCA	CATGATGTCC	CTGAGGTCCT	TGGGGGACAC
3651	GAAGGGCCCT	GCCAAGCTGC	TGTGGAAGGG	GGAGGGGGCT	GTGGTGATCC
	CTTCCCGGGA	CGGTTCGACG	ACÁCCTTCCC	CCTCCCCGA	CACCACTAGG
3701	AGGACAACTC	TGACATCAAG	GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC
	TCCTGTTGAG	ACTGTAGTTC	CACCACGGGT	CCTCCTTCCG	GTTCTAGTAG

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2002	GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAG	C
3801	CCTACTCCTG ATTTCGGGCC CGTCTAGACG ACACGGAAGA TCAACGGTC	'G
	CCTACTCCTG ATTICGGGCC GGTCTTGTG	
3851	CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGC	:C
3031	GTAGACAACA AACGGGGAGG GGGCACGGAA GGAACTGGGA CCTTCCACG	;G
3901	ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTC	T:
3701.	TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAG	£Α
3951	GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAG	3G
	CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTC	.C
		~m
4001	GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTC	- A
	CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAC	
	ATGGCCGATC GGCGCGCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGT	rg
4051	TACCGCTAG CCGCGCGCA TGACTTACA CACCCGCACC GAATTCCC	AC
	TACCGGCTAG CCGCGCGCA TGACTTIACT GITTOTT	
44.01	GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTT	ЗC
4101	CCTTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAA	CG
•		
4151	AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTTGATGGA AGCATTGT	3A
4101	TCGTCGGCGG CGGCGGTACT CGTGGTTGAG CAAACTACCT TCGTAACA	CT
4201	GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGA	ων Wî
	CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCT	• •
	GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCCCG CAAACTCT	AC
4251	GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCGGC GTTTGAGA CACTACCCGA GGTCGTAACT ACCAGCGGGG CAGGACGGGC GTTTGAGA	TG
	CACTACCCGA GGTCGTAACT ACCAGCGGGG CACGAGTGT	
4203	TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGC	CI
4301	ATGGAACTGG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCG	GA
4351	CCGCCGCCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTG	AC
4332	GGCGGCGGG AAGTCGGCGA CGTCGGTGGC GGGCGCCCTA ACACTGAC	TG:
4401	TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCC	:60
	AAACGAAAGG ACTCGGGCGA ACGTTTGTCA CGTCGAAGGG CAAGTAGG	ic G
	TO THE THE PROPERTY OF THE PRO	יככ
4451	CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACGGCGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTC	.cc 3GG
	GGCGCTACTG TTCAACTGCC GAGAAAACCG IGITAACCTA	
	GGGAACTTAA TGTCGTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGC	3TT
4501	CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTCGTCC	CAA
4551	TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAA	AA1
4331	AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTT	TTA
4601	AAAACCAGAC TCTGTTTGGA TTTGGATCAA GCAAGTGTCT TGCTGTC	TTT
,,,,	TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAG	AAA
4651	ATTTAGGGGT TTTGCGCGCG CGGTAGGCCC GGGACCAGCG GTCTCGG	7.CC
	TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTCGC CAGAGCC	AUC.

Figure 26E

4751	GTTCAGATAC	ATGGGCATAA	GCCCGTCTCT	GGGGTGGAGG	TAGCACCACT
	•			CCCCACCTCC	
4801	GCAGAGCTTC	ATGCTGCGGG	GTGGTGTTGT	AGATGATCCA	GTCGTAGCAG
	CGTCTCGAAG	TACGACGCCC	CACCACAACA	TCTACTAGGT	CAGCATCGTC
4851	GAGCGCTGGG	CGTGGTGCCT	AAAAATGTCT	TTCAGTAGCA	AGCTGATTGC
	CTCGCGACCC	GCACCACGGA	TTTTTACAGA	AAGTCATCGT	TCGACTAACG
4901	CAGGGGCAGG	CCCTTGGTGT	AAGTGTTTAC	AAAGCGGTTA	AGCTGGGATG
	GTCCCCGTCC	GGGAACCACA	TTCACAAATG	TTTCGCCAAT	TCGACCCTAC
4951	GGTGCATACG	TGGGGATATG	AGATGCATCT	TGGACTGTAT	TTTTAGGTTG
	CCACGTATGC	ACCCCTATAC	TCTACGTAGA	ACCTGACATA	AAAATCCAAC
5001	GCTATGTTCC	CAGCCATATC	CCTCCGGGGA	TTCATGTTGT	GCAGAACCAC
	CGATACAAGG	GTCGGTATAG	GGAGGCCCCT	AAGTACAACA	CGTCTTGGTG
5051	CAGCACAGTG	TATCCGGTGC	ACTTGGGAAA	TTTGTCATGT	AGCTTAGAAG
	GTCGTGTCAC	ATAGGCCACG	TGAACCCTTT	AAACAGTACA	TCGAATCTTC
5101	GAAATGCGTG	GAAGAACTTG	GAGACGCCCT	TGTGACCTCC	AAGATTTTCC
				ACACTGGAGG	
5151	ATGCATTCGT	CCATAATGAT	GGCAATGGGC	CCACGGGCGG	CGGCCTGGGC
	TACGTAAGCA	GGTATTACTA	CCGTTACCCG	GGTGCCCGCC	GCCGGACCCG
5201	GAAGATATTT	CTGGGATCAC	TAACGTCATA	GTTGTGTTCC	AGGATGAGAT
	CITCTATAAA	GACCCTAGTG	ATTGCAGTAT	CAACACAAGG	TCCTACTCTA
5251	CGTCATAGGC	CATTTTTACA	AAGCGCGGGC	GGAGGGTGCC	AGACTGCGGT
				CCTCCCACGG	
5301	ATAATGGTTC	CATCCGGCCC	AGGGGCGTAG	TTACCCTCAC	AGATTTGCAT
				AATGGGAGTG	
5351	TTCCCACGCT	TTGAGTTCAG	ATGGGGGGAT	CATGTCTACC	TGCGGGGCGA
				GTACAGATGG	
5401	TGAAGAAAAC	GGTTTCCGGG	GTAGGGGAGA	TCAGCTGGGA	AGAAAGCAGG
				AGTCGACCCT	
5451	TTCCTGAGCA	GCTGCGACTT	ACCGCAGCCG	GTGGGCCCGT	AAATCACACC
				CACCCGGGCA	
5501	TATTACCGGC	TGCAACTGGT	AGTTAAGAGA	GCTGCAGCTG	CCGTCATCCC
	•••			CGACGTCGAC	
5551	TGAGCAGGGG	GGCCACTTCG	TTAAGCATGT	CCCTGACTCG	CATGTTTTCC
				GGGACTGAGC	
5601	CTGACCAAAT	CCGCCAGAAG	GCGCTCGCCG	CCCAGCGATA	GCAGTTCTTG
	GACTGGTTTA	GGCGGTCTTC	CGCGAGCGGC	GGGTCGCTAT	CGTCAAGAAC

Figure 26 F

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£701	TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCCCACAG CTCGGTCAC	C.
5701	AAAACTCGCA AACTGGTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTC	G
5751	TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGC	.C
	ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCC	
	CGGCTTTCGC TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGC	3T
5801	GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCC	CA
5851	CATGTCTTTC CACGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTCACC	3G
3631	GTACAGAAAG GTGCCCGCGT CCCAGGAGCA GTCGCATCAG ACCCAGTG	CC
5901	TGAAGGGTG CGCTCCGGGC TGCGCGCTGG CCAGGGTGCG CTTGAGGC	, C
	ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCCACGC GAACTCCG	30
	GTCCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCCTGCG CGTCGGCC	AG
5951	CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGG	TC
6001	GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCC	CT
0001	CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACCGG	GA
6051	TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCA	CT.
	ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGT	C 1
	CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAG	TA
6101	GAAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGCCCCTC	AT
6151	GGCATCCGCG CCGCAGGCCC CGCAGACGGT CTCGCATTCC ACGAGCCA	.GG
6131	CCGTAGGCGC GGCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGT	CC.
6201	TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTTCCCCC ATGCTTT	J.C
	ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAA	LAC.
	ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GCTCGGTC	AC
6251	ATGCGTTTCT TACCTCTGGT TICCATGAGC CGGTGTCCAC TACGCAAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCAC	TG
6301	GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCCTC	CGA
6301	CTTTTCCGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAC	CT
•		
6351	GCGGTGTTCC GCGGTCCTCC TCGTATAGAA ACTCGGACCA CTCTGAG	ACA
	CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTC	IG1
	ACCOUNT OF THE PROPERTY OF THE	SCG
6401	AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTA TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTCACCC TCCCCAT	CGC
- 4 - 5	GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTGA AGACACA	TGT
6451	CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACACT TCTGTGT.	ACA
6501	CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCC	ACG
0301	GCGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGG	TGC
6551	TGACCGGGTG TTCCTGAAGG GGGGCTATAA AAGGGGGTGG GGGCGCG	AAG
	ACTGGCCCAC AAGGACTTCC CCCCGATATT TTCCCCCACC CCCGCGC	

Figure 266

6651	AGTACTCCCT	CTGAAAAGCG	GGCATGACTT	CTGCGCTAAG	ATTGTCAGTT
	TCATGAGGGA	GACTTTTCGC	CCGTACTGAA	GACGCGATTC	TAACAGTCAA
6701	TCCAAAAACG	AGGAGGATTT	GATATTCACC	TGGCCCGCGG	TGATGCCTTT
	AGGTTTTTGC	TCCTCCTAAA	CTATAAGTGG	ACCGGGCGCC	ACTACGGAAA
6751	GAGGGTGGCC	GCATCCATCT	GGTCAGAAAA	GACAATCTTT	TTGTTGTCAA
	CTCCCACCGG	CGTAGGTAGA	CCAGTCTTTT	CTGTTAGAAA	AACAACAGTT
6801	GCTTGGTGGC	AAACGACCCG	TAGAGGGCGT	TGGACAGCAA	CTTGGCGATG
	CGAACCACCG	TTTGCTGGGC	ATCTCCCGCA	ACCTGTCGTT	GAACCGCTAC
6851	GAGCGCAGGG	TTTGGTTTTT	GTCGCGATCG	GCGCGCTCCT	TGGCCGCGAT
	CTCGCGTCCC	AAACCAAAAA	CAGCGCTAGC	CGCGCGAGGA	ACCGGCGCTA
6901	GTTTAGCTGC	ACGTATTCGC	GCGCAACGCA	CCGCCATTCG	GGAAAGACGG
	CAAATCGACG	TGCATAAGCG	CGCGTTGCGT	GGCGGTAAGC	CCTTTCTGCC
6951	TGGTGCGCTC	GTCGGGCACC	AGGTGCACGC	GCCAACCGCG	GTTGTGCAGG
	ACCACGCGAG	CAGCCCGTGG	TCCACGTGCG	CGGTTGGCGC	CAACACGTCC
7001	GTGACAAGGT	CAACGCTGGT	GGCTACCTCT	CCGCGTAGGC	GCTCGTTGGT
	CACTGTTCCA	GTTGCGACCA	CCGATGGAGA	GGCGCATCCG	CGAGCAACCA
7051	CCAGCAGAGG	CGGCCGCCCT	TGCGCGAGCA	GAATGGCGGT	AGGGGGTCTA
	GGTCGTCTCC	GCCGGCGGGA	ACGCGCTCGT	CTTACCGCCA	TCCCCCAGAT
7101	GCTGCGTCTC CGACGCAGAG	GTCCGGGGGG CAGGCCCCCC	TCTGCGTCCA AGACGCAGGT	CGGTAAAGAC GCCATTTCTG	CCCGGGCAGC
7151	AGGCGCGCGT	CGAAGTAGTC	TATCTTGCAT	CCTTGCAAGT	CTAGCGCCTG
	TCCGCGCGCA	GCTTCATCAG	ATAGAACGTA	GGAACGTTCA	GATCGCGGAC
7201	CTGCCATGCG	CGGGCGGCAA	GCGCGCGCTC	GTATGGGTTG	AGTGGGGGAC
	GACGGTACGC	GCCCGCCGTT	CGCGCGCGAG	CATACCCAAC	TCACCCCCTG
7251	CCCATGGCAT	GGGGTGGGTG	AGCGCGGAGG	CGTACATGCC	GCAAATGTCG
	GGGTACCGTA	CCCCACCCAC	TCGCGCCTCC	GCATGTACGG	CGTTTACAGC
7301	TAAACGTAGA	GGGGCTCTCT	GAGTATTCCA	AGATATGTAG	GGTAGCATCT
	ATTTGCATCT	CCCCGAGAGA	CTCATAAGGT	TCTATACATC	CCATCGTAGA
7351	TCCACCGCGG	ATGCTGGCGC	GCACGTAATC	GTATAGTTCG	TGCGAGGGAG
	AGGTGGCGCC	TACGACCGCG	CGTGCATTAG	CATATCAAGC	ACGCTCCCTC
7401	CGAGGAGGTC	GGGACCGAGG	TTGCTACGGG	CGGGCTGCTC	TGCTCGGAAG
	GCTCCTCCAG	CCCTGGCTCC	AACGATGCCC	GCCCGACGAG	ACGAGCCTTC
7451	ACTATCTGCC	TGAAGATGGC	ATGTGAGTTG	GATGATATGG	TTGGACGCTG
	TGATAGACGG	ACTTCTACCG	TACACTCAAC	CTACTATACC	AACCTGCGAC
7501	GAAGACGTTG	AAGCTGGCGT	CTGTGAGACC	TACCGCGTCA	CGCACGAAGG
	CTTCTGCAAC	TTCGACCGCA	GACACTCTGG	ATGGCGCAGT	GCGTGCTTCC

Figure 26 H

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7601	TCTAGGGCGC	AGTAGTCCAG	GGTTTCCTTG	ATGATGTCAT	ACTTATCCTG
,002	AGATCCCGCG	TCATCAGGTC	CCAAAGGAAC	TACTACAGTA	TGAATAGGAC
7651	TCCCTTTTTT	TTCCACAGCT	CGCGGTTGAG	GACAAACTCT	TCGCGGTCTT
	AGGGAAAAA	AAGGTGTCGA	GCGCCAACTC	CTGTTTGAGA	AGCGCCAGAA
7701	TCCAGTACTC	TTGGATCGGA	AACCCGTCGG	CCTCCGAACG	GTAAGAGCCT
,,,,,	AGGTCATGAG	AACCTAGCCT	TTGGGCAGCC	GGAGGCTTGC	CATTCTCGGA
7751	AGCATGTAGA	ACTGGTTGAC	GGCCTGGTAG	GCGCAGCATC	CCTTTTCTAC
		TGACCAACTG			
7801	GGGTAGCGCG	TATGCCTGCG	CGGCCTTCCG	GAGCGAGGTG	TGGGTGAGCG
		ATACGGACGC			
7851	CAAAGGTGTC	CCTGACCATG	ACTTTGAGGT	ACTGGTATTT	GAAGTCAGTG
		GGACTGGTAC			
7901	TCGTCGCATC	CGCCCTGCTC	CCAGAGCAAA	AAGTCCGTGC	GCTTTTTGGA
		GCGGGACGAG			
7951	ACGCGGATTT	GGCAGGGCGA	AGGTGACATC	GTTGAAGAGT	TACAAAGGC
		CCGTCCCGCT			
8001	CGCGAGGCAT	AAAGTTGCGT	GTGATGCGGA	#CCCACCCC	CTCCOCACTTT
		TTTCAACGCA			
8051	CGGTTGTTAA	TTACCTGGGC	GGCGAGCACG	MACACCACTT	TCCCCAACTA
		AATGGACCCG			
8101	GTTGTGGCCC	ACAATGTAAA	GTTCCAAGAA	CCCCCCCTAC	CCCTTGATGG
		TGTTACATTT			
8151	AAGGCAATTT	TTTAAGTTCC	TUGTAGGTGA	CCACAAGTCC	GGAGCTGAGC
					AAGCGACGAA
8201	CCGTGCTCTG	AAAGGGCCCA	CACACGTTCT	COACCOARCE	TTCGCTGCTT
	GGCACGAGAC	111000001	Chancolle.		
B251	техестеско	AGGTCACGGG	CCATTAGCAT	TTGCAGGTGG	TCGCGAAAGG
	ACTCGAGGTG	TCCAGTGCCC	GGTAATCGT	AACGTCCACC	AGCGCTTTCC
8301	ήςςταλλςΤά	GCGACCTATO	GCCATTTTT	CTGGGGTGAT	GCAGTAGAAG
	AGGATTTGAC	CGCTGGATAC	CGGTAAAAA	GACCCCACTA	CGTCATCTTC
8351	GTAAGCGGGT	CTTGTTCCCA	GCGGTCCCA	CCAAGGTTC	CGGCTAGGTC
	CATTCGCCC	GAACAAGGG	CGCCAGGGT	A GGTTCCAAG(GCCGATCCAG
8401	TCGCGCGGCI	GTCACTAGAC	GCTCATCTC	C GCCGAACTTY	ATGACCAGCA
	AGCGCGCCG	CAGTGATCT	CGAGTAGAG	G CGGCTTGAA	TACTGGTCGT
8451	TGAAGGGCAG	GAGCTGCTT	CCAAAGGCC	C CCATCCAAG	TATAGGTCTCT
	ACTTCCCGT	CTCGACGAA	GGTTTCCGG	G GGTAGGTTC	A TATCCAGAGA

Figure 26 I 63/144

8551	GAAGAACTGG	ATCTCCCGCC	ACCAATTGGA	GGAGTGGCTA	TTGATGTGGT
	CTTCTTGACC	TAGAGGGCGG	TGGTTAACCT	CCTCACCGAT	AACTACACCA
8601	GAAAGTAGAA	GTCCCTGCGA	CGGGCCGAAC	ACTCGTGCTG	GCTTTTGTAA
	CTTTCATCTT	CAGGGACGCT	GCCCGGCTTG	TGAGCACGAC	CGAAAACATT
8651	AAACGTGCGC	AGTACTGGCA	GCGGTGCACG	GGCTGTACAT	CCTGCACGAG
	TTTGCACGCG	TCATGACCGT	CGCCACGTGC	CCGACATGTA	GGACGTGCTC
8701	GTTGACCTGA	CGACCGCGCA	CAAGGAAGCA	GAGTGGGAAT	TTGAGCCCCT
	CAACTGGACT	GCTGGCGCGT	GTTCCTTCGT	CTCACCCTTA	AACTCGGGGA
8751	CGCCTGGCGG	GTTTGGCTGG	TGGTCTTCTA	CTTCGGCTGC	TTGTCCTTGA
	GCGGACCGCC	CAAACCGACC	ACCAGAAGAT	GAAGCCGACG	AACAGGAACT
8801	CCGTCTGGCT	GCTCGAGGGG	AGTTACGGTG	GATCGGACCA	CCACGCCGCG
	GGCAGACCGA	CGAGCTCCCC	TCAATGCCAC	CTAGCCTGGT	GGTGCGGCGC
8851	CGAGCCCAAA GCTCGGGTTT	GTCCAGATGT CAGGTCTACA	ccccccccc	CGGTCGGAGC GCCAGCCTCG	TTGATGACAA AACTACTGTT
8901	CATCGCGCAG	ATGGGAGCTG	TCCATGGTCT	GGAGCTCCCG	CGGCGTCAGG
	GTAGCGCGTC	TACCCTCGAC	AGGTACCAGA	CCTCGAGGGC	GCCGCAGTCC
8951	TCAGGCGGGA	GCTCCTGCAG	GTTTACCTCG	CATAGACGGG	TCAGGGCGCG
	AGTCCGCCCT	CGAGGACGTC	CAAATGGAGC	GTATCTGCCC	AGTCCCGCGC
9001	GGCTAGATCC	AGGTGATACC	TAATTTCCAG	GGGCTGGTTG	GTGGCGGCGT
	CCGATCTAGG	TCCACTATGG	ATTAAAGGTC	CCCGACCAAC	CACCGCCGCA
9051	CGATGGCTTG	CAAGAGGCCG	CATCCCCGCG	GCGCGACTAC	GGTACCGCGC
	GCTACCGAAC	GTTCTCCGGC	GTAGGGGCGC	CGCGCTGATG	CCATGGCGCG
9101	GGCGGGCGGT CCGCCCGCCA	CCCGGCGCCC	GGTGTCCTTG CCACAGGAAC	GATGATGCAT CTACTACGTA	CTAAAAGCGG GATTTTCGCC
9151	TGACGCGGGC	GAGCCCCCGG	AGGTAGGGGG	GGCTCCGGAC	CCGCCGGGAG
	ACTGCGCCCG	CTCGGGGGCC	TCCATCCCCC	CCGAGGCCTG	GGCGGCCCTC
9201	AGGGGGCAGG TCCCCCGTCC	GGCACGTCGG CCGTGCAGCC	CCCCCCCCC	GGGCAGGAGC CCCGTCCTCG	TGGTGCTGCG ACCACGACGC
9251	CGCGTAGGTT	GCTGGCGAAC	GCGACGACGC	GGCGGTTGAT	CTCCTGAATC
	GCGCATCCAA	CGACCGCTTG	CGCTGCTGCG	CCGCCAACTA	GAGGACTTAG
9301	TGGCGCCTCT	GCGTGAAGAC	GACGGGCCCG	GTGAGCTTGA	ACCTGAAAGA
	ACCGCGGAGA	CGCACTTCTG	CTGCCCGGGC	CACTCGAACT	TGGACTTTCT
9351	GAGTTCGACA	GAATCAATTT	CGGTGTCGTT	GACGGCGGCC	TGGCGCAAAA
	CTCAAGCTGT	CTTAGTTAAA	GCCACAGCAA	CTGCCGCCGG	ACCGCGTTTT
9401	TCTCCTGCAC	GTCTCCTGAG	TTGTCTTGAT	AGGCGATCTC	GGCCATGAAC
	AGAGGACGTG	CAGAGGACTC	AACAGAACTA	TCCGCTAGAG	CCGGTACTTG

Figure 26 J

				as composition	AACCCCTTGA
9501	GGCGGCGAGG 1	CGTTGGAAA	TGCGGGCCAT	GAGC IGCGAG	TOTO COLD ACT
	CCGCCGCTCC)	GCAACCTTT	ACGCCCGGTA	CTCGACGCIC	110000.2.00
	GGCCTCCCTC (משתר בא הארם	CGGCTGTAGA	CCACGCCCCC	TTCGGCATCG
9551	CCGGAGGGAG (311CCAGACG	CCCCACATCT	GCTGCGGGG	AAGCCGTAGC
9601	CGGGCGCGCA '	TGACCACCTG	CGCGAGATTG	AGCTCCACGT	GCCGGGCGAA
3001	GCCCGCGCGT	ACTGGTGGAC	GCGCTCTAAC	TCGAGGTGCA	CGGCCCGCTT
0.051	GACGGCGTAG '	TTTCGCAGGC	GCTGAAAGAG	GTAGTTGAGG	GTGGTGGCGG
9651	CTGCCGCATC	DOOTTOOG	CGACTTTCTC	CATCAACTCC	CACCACCGCC
	TGTGTTCTGC	CACGAAGAAG	TACATAACCC	AGCGTCGCAA	CGTGGATTCG
9701	ACACAAGACG	CACCATOLIC	ATGTATTGGG	TCGCAGCGTT	GCACCTAAGC
	TTGATATCCC	רר א אכיברריתר	AAGGCGCTCC	ATGGCCTCGT	AGAAGTCCAC
9751	AACTATAGGG	CCAAGGCCIC	TTCCGCGAGG	TACCGGAGCA	TCTTCAGGTG
	GGCGAAGTTG	****	*GTTGCGCGC	CGACACGGTT	AACTCCTCCT
9801	CCGCTTCAAC	######C1GGG	TCAACGCGGG	GCTGTGCCAA	TTGAGGAGGA
	CCGCTTCAAC	TITITIGACCC	70,21000000		
	CCAGAAGACG		CCGACAGTGT	CGCGCACCTC	GCGCTCAAAG
9851	CCAGAAGACG GGTCTTCTGC	GATGAGCTCG	CCCTCTCACACA	GCGCGTGGAG	CGCGAGTTTC
	GGTCTTCTGC	CTACTCGAGC	CGC1G1Ci.c.		
	GCTACAGGGG		ምምር ምምር <u>ል</u> ልጥር	TCCTCTTCCA	TAAGGGCCTC
9901	GCTACAGGGG	CCTCTTCTTC	. IICIICAAAC	ACCAGAAGGT	ATTCCCGGAG
	CGATGTCCCC	GGAGAAGAAG	MAGAAGIIAC	, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
	CCCTTCTTCT		COCCTCCCC	AGGGGGGACA	CGGCGGCGAC
9951	CCCTTCTTCT	TCTTCTGGCG	, GCGG1GGGC	TOCCCCTGT	GCCGCCGCTG
	GGGAAGAAGA	AGAAGACCGC	COCCACCOC		
			- TCC2C22AG	GCTCGATCA	CTCCCCGGG
10001	GACGGCGCAC	CGGGAGGCGC	, YCCACAAA.C.	CGAGCTAGT	A GAGGGGGCGCC
	CTGCCGCGTG	GCCCTCCGC	, AGC1G111C	J Ca. 100 3 1 1 1 1	
			י כאררפרפרפ	c CCGTTCTCG	GGGGGGGCAG
10051	CGACGCCCA	TGGTCTCGG.	CACCCCCCCC	C GGCAAGAGC	CCCCCGCGTC
	GCTGCCGCGT	ACCAGAGCC	4 CIGCCGCGC	C 000:1:0:00	
			* mcmccccc	т атсссттсс	CCCCCGACG
10101	TTCGAAGACG	CCGCCCGTC	a rerecees	A TACCCAACC	G CCCCCGACG
	AACCTTCTGC	GGCGGCAG	1 ACAGGGCCA	A INCCOMICE	
				c አጥርጥሮልልሮል	A TTGTTGTGTA
10151	CATGCGGCAG	GGATACGGC	G CTAACGATG	C TACACTTGT	A TTGTTGTGTA
	GTACGCCGTC	CCTATGCCG	C GATTGCTAC	G INGRETTO:	T AACAACACAT
				C MCCCCATCG	A CCGGATCGGA
10201	GGTACTCCGC	CGCCGAGGG	A CCTGAGCGA	C ACCCCTAGC	A CCGGATCGGA
	CCATGAGGCG	GCGGCTCCC	T GGACICGUI	C MGGCGINGC	T GGCCTAGCCT
					A CCTACCCTGA
10251	AAACCTCTCG	AGAAAGGCG	T CTAACCAG	C MCMG1CGCA	A GGTAGGCTGA
	TTTGGAGAGC	TCTTTCCGC	A GATIGGICA	G TGTCAGCG1	T CCATCCGACT
10301	GCACCGTGGC	GGGCGGCAG	C GGGCGGCGC	71 CGGGGTTGT	T TCTGGCGGAG
	CGTGGCACC	CCCGCCGTC	G CCCGCCGC	A GCCCCAACA	A AGACCGCCTC
10351	GTGCTGCTG	TGATGTAA1	T AAAGTAGG	CG GTCTTGAGA	AC GGCGGATGGT
	CACGACGAC'	T ACTACATTA	A TITCATCC	SC CAGAACTC	NG CCGCCTACCA

Figure 26 K

10451				GGCGCAGGTC CCGCGTCCAG	
10501				TCTTCTCCTT AGAAGAGGAA	
10551				GGCGGAGTTT CCGCCTCAAA	
10601				CGAAGCCCCT GCTTCGGGGA	
10651				GCTAATATGG CGATTATACC	
10701	CTGCGTGAGG , GACGCACTCC			GTCCACAAAG CAGGTGTTTC	
10751				CCATAACGGA GGTATTGCCT	
10801				TACCTGAGAC ATGGACTCTG	
10851				CCGCACCAGG GGCGTGGTCC	
10901	CCACCAAAA GGTGGTTTTT	GTGCGGCGGC CACGCCGCCG	GGCTGGCGGT CCGACCGCCA	AGAGGGGCCA TCTCCCCGGT	GCGTAGGGTG CGCATCCCAC
10951	GCCGGGGCTC CGGCCCCGAG	CGGGGGCGAG GCCCCCGCTC	ATCTTCCAAC TAGAAGGTTG	ATAAGGCGAT TATTCCGCTA	GATATCCGTA CTATAGGCAT
11001				GGCGGTGGTG CCGCCACCAC	
11051	GAAAGTCGCG CTTTCAGCGC	GACGCGGTTC CTGCGCCAAG	CAGATGTTGC GTCTACAACG	GCAGCGGCAA CGTCGCCGTT	AAAGTGCTCC TTTCACGAGG
11101	ATGGTCGGGA TACCAGCCCT	CGCTCTGGCC GCGAGACCGG	GGTCAGGCGC CCAGTCCGCG	GCGCAATCGT CGCGTTAGCA	TGACGCTCTA ACTGCGAGAT
11151	GACCGTGCAA CTGGCACGTT	AAGGAGAGCC TTCCTCTCGG	TGTAAGCGGG ACATTCGCCC	CACTCTTCCG GTGAGAAGGC	TGGTCTGGTG ACCAGACCAC
11201	GATAAATTCG CTATTTAAGC	CAAGGGTATC GTTCCCATAG	ATGGCGGACG TACCGCCTGC	ACCGGGGTTC TGGCCCCAAG	GAGCCCCGTA CTCGGGGCAT
11251	TCCGGCCGTC AGGCCGGCAG	CGCCGTGATC GCGGCACTAG	CATGCGGTTA GTACGCCAAT	CCGCCCGCGT	GTCGAACCCA CAGCTTGGGT
11301	GGTGTGCGAC CCACACGCTG	GTCAGACAAC CAGTCTGTTG	GGGGGAGTGC CCCCTCACG	TCCTTTTGGC AGGAAAACCG	TTCCTTCCAG AAGGAAGGTC

Figure 26L

11401	AACCGGTTAG	GCTGGAAAGC	GAAAGCATTA	AGTGGCTCGC	TCCCTGTAGC
11401	TTCGCCAATC	CGACCTTTCG	CTTTCGTAAT	TCACCGAGCG	AGGGACATCG
11451	CGGAGGGTTA	TTTTCCAAGG	GTTGAGTCGC	GGGACCCCCG	GTTCGAGTCT
	GCCTCCCAAT	AAAAGGTTCC	CAACTCAGCG	CCCTGGGGGC	CAAGCTCAGA
11501	CGGACCGGCC	GGACTGCGGC	GAACGGGGGT	TTGCCTCCCC	GTCATGCAAG
		•	CTTGCCCCCA		
11551	ACCCCGCTTG	CAAATTCCTC	CGGAAACAGG	GACGAGCCCC	TTTTTTGCTT
		•	GCCTTTGTCC		
11601	TTCCCAGATG	CATCCGGTGC	TGCGGCAGAT	GCGCCCCCT	CCTCAGCAGC
			ACGCCGTCTA		
11651	GGCAAGAGCA	AGAGCAGCGG	CAGACATGCA	GGGCACCCTC	CCCACCACCA
			GTCTGTACGT		
11701	ACCGCGTCAG	GAGGGGCGAC	ATCCGCGGTT	CACGCGGCAG	CAGAIGGIGA
			TAGGCGCCAA		
11751	TTACGAACCC	CCGCGGCGCC	GGGCCCGGCA	CTACCTGGAC	TTGGAGGAGG
			CCCGGGCCGT		
11801	GCGAGGGCCT	GGCGCGGCTA	GGAGCGCCCT	CTCCTGAGCG	GCACCCAAGG
			CCTCGCGGGA		
11851	GTGCAGCTGA	AGCGTGATAC	GCGTGAGGCG	TACGTGCCGC	CCCCCTTCGA
			CGCACTCCGC		
11901	GTTTCGCGAC	CGCGAGGGAG	AGGAGCCCGA	GGAGATGCGG	GATCGAAAGT
					CTAGCTTTCA
11951	TCCACGCAGG	GCGCGAGCTG	CGGCATGGCC	TGAATCGCGA	CCCCAACGAC
					CGCCAACGAC
12001	CGCGAGGAGG	ACTTTGAGCC	CGACGCGCGA	ACCGGGATTA	DODODOOOTO
					CAGGGCGCGC
12051	CGCACACGTG	GCGGCCGCCG	ACCTGGTAAC	CGCATACGAG	CAGACGGTGA
					GTCTGCCACT
12101	ACCAGGAGAT	TAACTTTCA	AAAAGCTTTA	ACAACCACG	TTODOATDOD 1
	TGGTCCTCTA	ATTGAAAGTT	TTTTCGAAAI	TGTTGGTGC	CGCAIGCGAA
12151	GTGGCGCGCC	AGGAGGTGG	TATAGGACTO	ATGCATCTG	r GGGACTTTGT
	CACCGCGCGC	TCCTCCACC	S ATATCCTGAC	TACGTAGACA	CCCIONNEX
12201	AAGCGCGCTC	GAGCAAAAC	CAAATAGCAA	GCCGCTCAT	GCGCAGCTGT
	TTCGCGCGA	CTCGTTTTG	G GTTTATCGT1	r CGGCGAGTA	CGCGTCGACA
12251	תרׁריתיאַ יוֹאַריִי יוֹם אַ דיים אַריים אַריים אַריים אַריים אַריים אַריים אַריים אַריים אַריים אַריים אַריים אַריים אַריים אַריים	r GCAGCACAG	AGGGACAAC	AGGCATTCA	G GGATGCGCTG
12431	AGGAATATC	A CGTCGTGTC	G TCCCTGTTG	TCCGTAAGT	C CCTACGCGAC

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12351	CCTGCAGAGC	ATAGTGGTGC	AGGAGCGCAG	CTTGAGCCTG	GCTGACAAGG
	GGACGTCTCG	TATCACCACG	TCCTCGCGTC	GAACTCGGAC	CGACTGTTCC
12401	TCCCCCCCAT	СВВСТВТТСС	ATGCTTAGCC	TGGGCAAGTT	TTACGCCCGC
12401	1000000000		MACCA ATCCC	ACCCGTTCAA	AATGCGGGCG
					•
12451	AAGATATACC	ATACCCCTTA	CGTTCCCATA	GACAAGGAGG	TAAAGATCGA
	ጥጥርጥልጥልጥርና	TATGGGGAAT	GCAAGGGTAT	CTGTTCCTCC	ATTTCTAGCT
12501	GGGGTTCTAC	ATGCGCATGG	CGCTGAAGGT	GCTTACCTTG	AGCGACGACC
	CCCCAAGATG	TACGCGTACC	GCGACTTCCA	CGAATGGAAC	TCGCTGCTGG
10551	MCCCCCTTT A	TOCOLACGAG	CCCATCCACA	AGGCCGTGAG	CGTGAGCCGG
12551	TGGGCGIIIA	1CGCAACGAG	CGCA1CCACA	TCCGGCACTC	CCACTCGGCC
12601	CGGCGCGAGC	TCAGCGACCG	CGAGCTGATG	CACAGCCTGC	AAAGGGCCCT
12001	CCCCCCCCCCC	ACTOCOTOGO	GCTCGACTAC	GTGTCGGACG	TTTCCCGGGA
12651	GGCTGGCACG	GGCAGCGGCG	ATAGAGAGGC	CGAGTCCTAC	TTTGACGCGG
	CCGACCGTGC	CCGTCGCCGC	TATCTCTCCG	GCTCAGGATG	AAACTGCGCC
		0000000000	CCARCCCCAC	GCGCCCTGGA	GGCAGCTGGG
12701	GCGCTGACCT	100000000000000000000000000000000000000	CCAAGCCGAC	CCCCCCACC	CCCTCCACCC
	CGCGACTGGA	CGCGACCCGG	GGTTCGGCTG	CGCGGGACCT	CCG1CGACCC
12751	CCCCCACCTG	GCTGGCGGT	GGCACCCGCG	CGCGCTGGCA	ACGTCGGCGG
12/31	CCCCCTCCAC	CCCACCCCCA	СССТСССССС	GCGCGACCGT	TGCAGCCGCC
12801	CGTGGAGGAA	TATGACGAGG	ACGATGAGTA	CGAGCCAGAG	GACGGCGAGT
	GCACCTCCTT	ATACTGCTCC	TGCTACTCAT	GCTCGGTCTC	CTGCCGCTCA
12051	> CEN > CCCCT	こゝかごかかかごひこ	ATCAGATGAT	GCAAGACGCA	ACGGACCCGG
12851	ACTAMGCGGT	CMACAAACAC	TACTOTACTA	CGTTCTGCGT	TECCTEGECC
12901	CGGTGCGGGC	GGCGCTGCAG	AGCCAGCCGT	CCGGCCTTAA	CTCCACGGAC
	GCCACGCCCG	CCGCGACGTC	TCGGTCGGCA	GGCCGGAATT	GAGGTGCCTG
	63 0BCCCCCC	א כיכיזיר א זייניניא	CCCCATCATG	TCGCTGACTG	CGCGCAATCC
12951	GACIGGCGCC	MOGICATOON	CCCCTACTAC	AGCGACTGAC	CCCCCTTAGG
13001	TGACGCGTTC	CGGCAGCAGC	CGCAGGCCAA	CCGGCTCTCC	GCAATTCTGG
15001	ACTGCGCAAG	GCCGTCGTCG	GCGTCCGGTT	GGCCGAGAGG	CGTTAAGACC
			0022200000	CCCACCAGAA	GGTGCTGGCG
13051	AAGCGGTGGT	CCCGGCGCGC	GCAAACCCCA	CGCACGAGAA	001001000
	TTCGCCACCA	GGGCCGCGCG	CGTTTGGGGT	GCGTGCTCTT	CCACGACCGC
12101	AUCCUPATOC	CCCTCCCCCCA	AAACAGGGCC	ATCCGGCCCG	ACGAGGCCGG
12101	VICGIVUVCG	CCCACCCCC	שייינייניינייניינייניינייניינייניינייניי	TAGGCCGGGC	TGCTCCGGCC
13151	CCTGGTCTAC	GACGCGCTGC	TTCAGCGCGT	GGCTCGTTAC	AACAGCGGCA
	GGACCAGATG	CTGCGCGACG	AAGTCGCGCA	CCGAGCAATG	TTGTCGCCGT
13201	ACGTGCAGAC	CAACCTGGAC	CGGCTGGTGG	GGGATGTGCG	CGAGGCCGTG
	ጥርር እ ርርጥርጥር	GTTGGACCTG	GCCGACCACC	CCCTACACGC	GCTCCGGCAC
	.GCACG1616				

Figure 26 N

13301	ACTAAACGCC	TTCCTGAGTA	CACAGCCCGC	CAACGTGCCG	CGGGGACAGG
	TGATTTGCGG	AAGGACTCAT	GTGTCGGGCG	GTTGCACGGC	GCCCTGTCC
13351	AGGACTACAC	CAACTTTGTG	AGCGCACTGC	GGCTAATGGT	GACTGAGACA
	TCCTGATGTG	GTTGAAACAC	TCGCGTGACG	CCGATTACCA	CTGACTCTGT
13401	CCGCAAAGTG	AGGTGTACCA	GTCTGGGCCA	GACTATTTTT	TCCAGACCAG
	GGCGTTTCAC	TCCACATGGT	CAGACCCGGT	CTGATAAAAA	AGGTCTGGTC
13451	TAGACAAGGC	CTGCAGACCG	TAAACCTGAG	CCAGGCTTTC	AAAAACTTGC
	ATCTGTTCCG	GACGTCTGGC	ATTTGGACTC	GGTCCGAAAG	TTTTTGAACG
13501	AGGGGCTGTG	GGGGGTGCGG	GCTCCCACAG	GCGACCGCGC	GACCGTGTCT
	TCCCCGACAC	CCCCCACGCC	CGAGGGTGTC	CGCTGGCGCG	CTGGCACAGA
13551	AGCTTGCTGA	CGCCCAACTC	GCGCCTGTTG	CTGCTGCTAA	TAGCGCCCTT
	TCGAACGACT	GCGGGTTGAG	CGCGGACAAC	GACGACGATT	ATCGCGGGAA
13601	CACGGACAGT	GGCAGCGTGT	CCCGGGACAC	ATACCTAGGT	CACTTGCTGA
	GTGCCTGTCA	CCGTCGCACA	GGGCCCTGTG	TATGGATCCA	GTGAACGACT
13651	CACTGTACCG	CGAGGCCATA	GGTCAGGCGC	ATGTGGACGA	GCATACTTTC
	GTGACATGGC	GCTCCGGTAT	CCAGTCCGCG	TACACCTGCT	CGTATGAAAG
13701	CAGGAGATTA	CAAGTGTCAG	CCGCGCGCTG	GGGCAGGAGG	ACACGGGCAG
	GTCCTCTAAT	GTTCACAGTC	GGCGCGCGAC	CCCGTCCTCC	TGTGCCCGTC
13751	CCTGGAGGCA	ACCCTAAACT	ACCTGCTGAC	CAACCGGCGG	CAGAAGATCC
	GGACCTCCGT	TGGGATTTGA	TGGACGACTG	GTTGGCCGCC	GTCTTCTAGG
13801	CCTCGTTGCA GGAGCAACGT	CAGTTTAAAC GTCAAATTTG	AGCGAGGAGG TCGCTCCTCC	AGCGCATTTT TCGCGTAAAA	GCGCTACGTG
13851	CAGCAGAGCG	TGAGCCTTAA	CCTGATGCGC	GACGGGGTAA	CGCCCAGCGT
	GTCGTCTCGC	ACTCGGAATT	GGACTACGCG	CTGCCCCATT	GCGGGTCGCA
13901	GGCGCTGGAC	ATGACCGCGC	GCAACATGGA	ACCGGGCATG	TATGCCTCAA
	CCGCGACCTG	TACTGGCGCG	CGTTGTACCT	TGGCCCGTAC	ATACGGAGTT
13951	ACCGGCCGTT TGGCCGGCAA	TATCAACCGC ATAGTTGGCG	CTAATGGACT GATTACCTGA	ACTTGCATCG TGAACGTAGG	: GCGCCGCCGCC
	CACTTGGGGC	TCATAAAGTG	GTTACGGTAG	AACTTGGGCG	: ACTGGCTACC : TGACCGATGG
	CGGGGGACCA	AAGATGTGGC	CCCCTAAGC7	CCACGGGCT	GGTAACGATG CCATTGCTAC
	CTAAGGAGAC	CCTGCTGTAT	CTGCTGTCG(CACAAAAGGGG	GCAACCGCAG GCGTTGGCGTC
14151	ACCCTGCTAC TGGGACGATC	AGTTGCAACA TCAACGTTG	CGCGCAGCA(CGCGCTCGT(GCAGAGGCG(GCGACGCTTT

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14251	CGCGGTCAGA	TGCTAGTAGC	CCATTTCCAA	GCTTGATAGG	GTCTCTTACC
	GCGCCAGTCT	ACGATCATCG	GGTAAAGGTT	CGAACTATCC	CAGAGAATGG
14301	AGCACTCGCA	CCACCGCCC	GCGCCTGCTG	GGCGAGGAGG	AGTACCTAAA
	TCGTGAGCGT	GGTGGGCGGG	CGCGGACGAC	CCGCTCCTCC	TCATGGATTT
14351	CAACTCGCTG	CTGCAGCCGC	AGCGCGAAAA	AAACCTGCCT	CCGGCATTTC
	GTTGAGCGAC	GACGTCGGCG	TCGCGCTTTT	TTTGGACGGA	GGCCGTAAAG
14401	CCAACAACGG	GATAGAGAGC	CTAGTGGACA	AGATGAGTAG	ATGGAAGACG
	GGTTGTTGCC	CTATCTCTCG	GATCACCTGT	TCTACTCATC	TACCTTCTGC
14451	TACGCGCAGG ATGCGCGTCC	AGCACAGGGA TCGTGTCCCT	CGTGCCAGGC GCACGGTCCG	CCGCGCCCGC	CCACCCGTCG GGTGGGCAGC
14501	TCAAAGGCAC	GACCGTCAGC	GGGGTCTGGT	GTGGGAGGAC	GATGACTCGG
	AGTTTCCGTG	CTGGCAGTCG	CCCCAGACCA	CACCCTCCTG	CTACTGAGCC
14551	CAGACGACAG	CAGCGTCCTG	GATTTGGGAG	GGAGTGGCAA	CCCGTTTGCG
	GTCTGCTGTC	GTCGCAGGAC	CTAAACCCTC	CCTCACCGTT	GGGCAAACGC
14601	CACCTTCGCC	CCAGGCTGGG	GAGAATGTTT	TAAAAAAAA	AAAAGCATGA
	GTGGAAGCGG	GGTCCGACCC	CTCTTACAAA	TTTTTTTTT	TTTTCGTACT
14651	TGCAAAATAA	AAAACTCACC	AAGGCCATGG	CACCGAGCGT	TGGTTTTCTT
	ACGTTTTATT	TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	GTATTCCCCT	TAGTATGCGG	CGCGCGGCGA	TGTATGAGGA	AGGTCCTCCT
	CATAAGGGGA	ATCATACGCC	GCGCGCCGCT	ACATACTCCT	TCCAGGAGGA
14751	CCCTCCTACG	AGAGTGTGGT	GAGCGCGGCG	CCAGTGGCGG	CGGCGCTGGG
	GGGAGGATGC	TCTCACACCA	CTCGCGCCGC	GGTCACCGCC	GCCGCGACCC
14801	TTCTCCCTTC	GATGCTCCCC	TGGACCCGCC	GTTTGTGCCT	CCGCGGTACC
	AAGAGGGAAG	CTACGAGGGG	ACCTGGGCGG	CAAACACGGA	GGCGCCATGG
14851	TGCGGCCTAC	CGGGGGGAGA	AACAGCATCC	GTTACTCTGA	GTTGGCACCC
	ACGCCGGATG	GCCCCCTCT	TTGTCGTAGG	CAATGAGACT	CAACCGTGGG
14901	CTATTCGACA	CCACCCGTGT	GTACCTGGTG	GACAACAAGT	CAACGGATGT
	GATAAGCTGT	GGTGGGCACA	CATGGACCAC	CTGTTGTTCA	GTTGCCTACA
14951	GGCATCCCTG	AACTACCAGA	ACGACCACAG	CAACTTTCTG	ACCACGGTCA
	CCGTAGGGAC	TTGATGGTCT	TGCTGGTGTC	GTTGAAAGAC	TGGTGCCAGT
15001	TTCAAAACAA	TGACTACAGC	CCGGGGGAGG	CAAGCACACA	GACCATCAAT
	AAGTTTTGTT	ACTGATGTCG	GGCCCCTCC	GTTCGTGTGT	CTGGTAGTTA
15051	CTTGACGACC	GGTCGCACTG	GGGCGGCGAC	CTGAAAACCA	TCCTGCATAC
	GAACTGCTGG	CCAGCGTGAC	CCCGCCGCTG	GACTTTTGGT	AGGACGTATG
15101	CAACATGCCA	AATGTGAACG	AGTTCATGTT	TACCAATAAG	TTTAAGGCGC
	GTTGTACGGT	TTACACTTGC	TCAAGTACAA	ATGGTTATTC	AAATTCCGCG

Figure 26 P

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15151		GTCGCGCTTG CAGCGCGAAC			
15201	TACGAGTGGG ATGCTCACCC				
15251	GACCATAGAC CTGGTATCTG	CTTATGAACA GAATACTTGT			
15301		CGGGGTTCTG GCCCCAAGAC			
15351	GCGTTGAAGT	GACTGGGGTT CTGACCCCAA	ACTGGGGCAG	TGACCAGAAC	AGTACGGACC
15401	CCATATATGT	AACGAAGCCT TTGCTTCGGA	AGGTAGGTCT	GTAGTAAAAC	GACGGTCCTA
15451	CGCCCCACCT	CTTCACCCAC GAAGTGGGTG	TCGGCGGACT	CGTTGAACAA	CCCGTAGGCG
15501	TTCGCCGTTG	CCTTCCAGGA GGAAGGTCCT	CCCGAAATCC	TAGTGGATGC	TACTAGACCT
15551	CCCACCATTG	ATTCCCGCAC TAAGGGCGTG	ACAACCTACA	CCTGCGGATG	GTCCGCTCGA
15601	ACTTTCTACT	CACCGAACAG GTGGCTTGTC	CCGCCCCCAC	CGCGTCCGCC	GTCGTTGTCG
15651	TCACCGTCGC	GCGCGGAAGA CGCGCCTTCT	CTTGAGGTTG	CGCCGTCGGC	GCCGTTACGT
15701	CGGCCACCTC	GACATGAACG CTGTACTTGC	TAGTACGGTA	AGCGCCGCTG	TGGAAACGGT
15751	GTGCCCGACT	GGAGAAGCGC CCTCTTCGCG	CGACTCCGGC	TTCGTCGCCG	GCTTCGACGG
15801	CGGGGGCGAC	CGCAACCCGA GCGTTGGGCT	CCAGCTCTTC	GGAGTCTTCT	TTGGCCACTA
		TGTCTCCTGT	CGTTCTTTGC	GTCAATGTTG	GATTATTCGT
		GAAGTGGGTC	ATGGCGTCGA	CCATGGAACG	TATGTTGATG
		TCTGGCCTTA	GGCGAGTACC	TGGGACGAAA	CGTGAGGACT
		CCGAGCCTCG	TCCAGATGAC	CAGCAACGGT	CTGTACTACG
16051	AAGACCCCGT TTCTGGGGCA	GACCTTCCGC CTGGAAGGCG	TCCACGCGCC AGGTGCGCGG	AGATCAGCAA TCTAGTCGTT	CTTTCCGGTG GAAAGGCCAC

Figure 26 Q

16151	GGCCGTCTAC	TCCCAACTCA	TCCGCCAGTT	TACCTCTCTG	ACCCACGTGT
	CCGGCAGATG	AGGGTTGAGT	AGGCGGTCAA	ATGGAGAGAC	TGGGTGCACA
16201				ececececec cecececec	
16251	ATCACCACCG	TCAGTGAAAA	CGTTCCTGCT	CTCACAGATC	ACGGGACGCT
	TAGTGGTGGC	AGTCACTTTT	GCAAGGACGA	GAGTGTCTAG	TGCCCTGCGA
16301	ACCGCTGCGC	AACAGCATCG	GAGGAGTCCA	GCGAGTGACC	ATTACTGACG
	TGGCGACGCG	TTGTCGTAGC	CTCCTCAGGT	CGCTCACTGG	TAATGACTGC
16351	CCAGACGCCG	CACCTGCCCC	TACGTTTACA	AGGCCCTGGG	CATAGTCTCG
	GGTCTGCGGC	GTGGACGGGG	ATGCAAATGT	TCCGGGACCC	GTATCAGAGC
16401	CCGCGCGTCC	TATCGAGCCG	CACTTTTTGA	GCAAGCATGT	CCATCCTTAT
	GGCGCGCAGG	ATAGCTCGGC	GTGAAAAACT	CGTTCGTACA	GGTAGGAATA
16451	ATCGCCCAGC	AATAACACAG	GCTGGGGCCT	GCGCTTCCCA	AGCAAGATGT
	TAGCGGGTCG	TTATTGTGTC	CGACCCCGGA	CGCGAAGGGT	TCGTTCTACA
16501	TTGGCGGGGC	CAAGAAGCGC	TCCGACCAAC	ACCCAGTGCG	CGTGCGCGGG
	AACCGCCCCG	GTTCTTCGCG	AGGCTGGTTG	TGGGTCACGC	GCACGCGCCC
16551	GTGATGGCGC	GCGGGACCCC	GCGCGTGTTT	CGCGGCCGCA GCGCCGCGT	GACCCGCGTG
16601	CACCGTCGAT	GACGCCATCG	ACGCGGTGGT	GGAGGAGGCG	CGCAACTACA
	GTGGCAGCTA	CTGCGGTAGC	TGCGCCACCA	CCTCCTCCGC	GCGTTGATGT
16651	GCGGGTGCGG	CGGTGGTCAC	AGGTGTCACC	ACGCGGCCAT TGCGCCGGTA	AGTCTGGCAC
16701	GTGCGCGGAG	CCCGGCGCTA	TGCTAAAATG	AAGAGACGGC	GGAGGCGCGT
	CACGCGCCTC	GGGCCGCGAT	ACGATTTTAC	TTCTCTGCCG	CCTCCGCGCA
16751	AGCACGTCGC TCGTGCAGCG	CACCGCCGCC GTGGCGGCGG	GACCCGGCAC CTGGGCCGTG	TGCCGCCCAA ACGGCGGGTT	CCCCCCCCC
16801	CGGCCCTGCT	TAACCGCGCA	CGTCGCACCG	GCCGACGGGC	GGCCATGCGG
	GCCGGGACGA	ATTGGCGCGT	GCAGCGTGGC	CGGCTGCCCG	CCGGTACGCC
16851	GCCGCTCGAA	GGCTGGCCGC	GGGTATTGTC	ACTGTGCCCC	CCAGGTCCAG
	CGGCGAGCTT	CCGACCGGCG	CCCATAACAG	TGACACGGGG	GGTCCAGGTC
16901	GCGACGAGCG CGCTGCTCGC	GCCGCCGCAG	CAGCCGCGGC	CATTAGTGCT GTAATCACGA	ATGACTCAGG TACTGAGTCC
16951	GTCGCAGGGG CAGCGTCCCC	CAACGTGTAT GTTGCACATA	TGGGTGCGCG ACCCACGCGC	ACTCGGTTAG TGAGCCAATC	CGGCCTGCGC
17001	GTGCCCGTGC	GCACCCGCCC	CCCGCGCAAC	TAGATTGCAA	GAAAAAACTA
	CACGGGCACG	CGTGGGCGGG	GGGCGCGTTG	ATCTAACGTT	CTTTTTTGAT

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17101	CTATGTCCAA	GCGCAAAATC	AAAGAAGAGA	TGCTCCAGGT	CATCGCGCCG
	GATACAGGTT	CGCGTTTTAG	${\tt TTTCTTCTCT}$	ACGAGGTCCA	GTAGCGCGGC
17151	GAGATCTATG	GCCCCCGAA	GAAGGAAGAG	CAGGATTACA	AGCCCCGAAA
	CTCTAGATAC	CGGGGGCTT	CTTCCTTCTC	GTCCTAATGT	TCGGGGCTTT
4 = 0.01	GCTAAAGCGG	CDC N N N N N C N	****	TCATCATCAT	GAACTTGACG
17201	CCARTTTCCCC	C) CAMPAGA	TTTTCTTTCT	ACTACTACTA	CTTGAACTGC
	CGATTICGCC	CAGIIIIICI			
17251	ACGAGGTGGA	ACTGCTGCAC	GCTACCGCGC	CCAGGCGACG	GGTACAGTGG
	TGCTCCACCT	TGACGACGTG	CGATGGCGCG	GGTCCGCTGC	CCATGTCACC
17301	AAAGGTCGAC	GCGTAAAACG	TGTTTTGCGA	CCCGGCACCA	CCGTAGTCTT
	TTTCCAGCTG	CGCATTTTGC	ACAAAACGCT	GGGCCGTGGT	GGCATCAGAA
			CCCGCACCTA	CANCCCCCTC	TATCATCACC
17351			GGGCGTGGAT		
	ATGCGGGCCA	CICGCGAGGI	GGGCGIGGAI	GIICOCOCAC	
17401	TGTACGGCGA	CGAGGACCTG	CTTGAGCAGG	CCAACGAGCG	CCTCGGGGAG
11401	ACATGCCGCT	GCTCCTGGAC	GAACTCGTCC	GGTTGCTCGC	GGAGCCCCTC
17451	TTTGCCTACG	GAAAGCGGCA	TAAGGACATG	CTGGCGTTGC	CGCTGGACGA
	AAACGGATGC	CTTTCGCCGT	ATTCCTGTAC	GACCGCAACG	GCGACCTGCT
			TAAAGCCCGT	*********	СУССТССТСС
17501	GGGCAACCCA	MCACCTAGCC	ATTTCGGGCA	TTGTGACGTC	GTCCACGACG
	CCCGTTGGGT	TGTGGATCGG	ATTICGGGCA	1101000010	G100.100
17551	CCCCCCTTGC	ACCGTCCGAA	GAAAAGCGCG	GCCTAAAGCG	CGAGTCTGGT
1/331	GGCGCGAACG	TGGCAGGCTT	CTTTTCGCGC	CGGATTTCGC	GCTCAGACCA
	_				
17601	GACTTGGCAC	CCACCGTGCA	GCTGATGGTA	CCCAAGCGCC	AGCGACTGGA
	CTGAACCGTG	GGTGGCACGT	CGACTACCAT	GGGTTCGCGG	TCGCTGACCT
				macaamaa k	CCCCACCTCC
17651	AGATGTCTTG	GAAAAAATGA	CCGTGGAACC GGCACCTTGG	ACCCCACCTC	GCCCACGICC
	TCTACAGAAC	CTTTTTACI	GGCACCIIGG	ACCCGACCIC	000010000
17701	CCCTCCCCC	AATCAAGCAG	GTGGCGCCGG	GACTGGGCGT	GCAGACCGTG
17701	CGCACGCCGG	TTAGTTCGTC	CACCGCGGCC	CTGACCCGCA	CGTCTGGCAC
17751	GACGTTCAGA	TACCCACTAC	CAGTAGCACC	AGTATTGCCA	CCGCCACAGA
•	CTGCAAGTCT	ATGGGTGATG	GTCATCGTGG	TCATAACGGT	GGCGGTGTCT
				CDC > CCCCTC	GCGGATGCCG
17801	GGGCATGGAG	ACACAAACGT	CCCCGGTTGC	CACTCCCCCC	CGCCTACGGC
	CCCGTACCTC	TOTOTTICCA	SOUS CANCE		
17951	CCCTCCACCC	GGTCGCTGCG	GCCGCGTCCA	AGACCTCTAC	GGAGGTGCAA
TIONI	GCCACGTCCG	CCAGCGACGC	CGGCGCAGGT	TCTGGAGATG	CCTCCACGTT
17901	ACGGACCCGT	GGATGTTTCG	CGTTTCAGCC	CCCCGGCGCC	CGCGCCGTTC
	TGCCTGGGCA	CCTACAAAGC	GCAAAGTCGG	GGGCCCCCG	GCGCGGCAAG
			000000000000000000000000000000000000000		- CCCCT3C3TC
17951	GAGGAAGTAC	GGCGCCGCCA	CCCCCCAMCA	CCCCCTTATA	GCCCTACATC CGGGATGTAG
	CTCCTTCATG		CGCGCGATGA	COGCIINIA	

Figure 265

18051	AGACGAGCAA TCTGCTCGTT	CTACCCGACG GATGGGCTGC	CCGAACCACC GGCTTGGTGG	ACTGGAACCC TGACCTTGGG	CGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
18101	TCGCCGTCGC AGCGGCAGCG	CAGCCCGTGC GTCGGGCACG	TGGCCCCGAT ACCGGGGCTA	TTCCGTGCGC AAGGCACGCG	AGGGTGGCTC TCCCACCGAG
18151	GCGAAGGAGG CGCTTCCTCC	CAGGACCCTG GTCCTGGGAC	GTGCTGCCAA CACGACGGTT	CAGCGCGCTA GTCGCGCGAT	CCACCCAGC GGTGGGGTCG
18201	ATCGTTTAAA TAGCAAATTT	AGCCGGTCTT TCGGCCAGAA	TGTGGTTCTT ACACCAAGAA	GCAGATATGG CGTCTATACC	CCCTCACCTG GGGAGTGGAC
18251	CCGCCTCCGT GGCGGAGGCA	TTCCCGGTGC AAGGGCCACG	CGGGATTCCG GCCCTAAGGC	AGGAAGAATG TCCTTCTTAC	CACCGTAGGA GTGGCATCCT
18301	CCCCGTACCG	CGGCCACGGC GCCGGTGCCG	GACTGCCCGC	CGTACGCAGC	ACGCGTGGTG
18351	GCCGCCGCCG	GCGCGTCGCA CGCGCAGCGT	GGCAGCGTAC	GCGCCGCCAT	AGGACGGGGA
18401	GGAATAAGGT	CTGATCGCCG GACTAGCGGC	GCCGCTAACC	GCGGCACGGG	CCTTAACGTA
18451	GGCACCGGAA	GCAGGCGCAG CGTCCGCGTC	TCTGTGACTA	ATTTTTGTTC	AACGTACACC
18501		TATTTTTCAG	ACCTGAGAGT	GCGAGCGAAC	CAGGACATTG
18551	ATAAAACATC	AATGGAAGAC TTACCTTCTG	TAGTTGAAAC	GCAGAGACCG	GGGCGCTGTG
18601	CCGAGCGCGG	CGTTCATGGG GCAAGTACCC GCCTTCAGCT	TTTGACCGTT	CTATAGCCGT	GGTCGTTATA
18651	CTCGCCACCG TCGGTTCCAC	CGGAAGTCGA	CCCCGAGCGA	CACCTCGCCG	TAATTTTTAA
	AGCCAAGGTG GGCCAGATGC	GCAATTCTTG	ATACCGTCGT	TCCGGACCTT	GTCGTCGTGT
	CCGGTCTACG	ACTCCCTATT	CAACTTTCTC	GTTTTAAAGG	TTGTTTTCCA
	CCATCTACCG AGGCAGTGCA	GACCGGAGAC	CGTAATCGCC	CCACCACCTG	GACCGGTTGG
	TCCGTCACGT GAGGAGCCTC	TTTATTCTAA	TTGTCATTCG	AACTAGGGGC	GGGAGGCAT
19201	CTCCTCGGAG	GTGGCCGGCA	CCTCTGTCAC	AGAGGTCTCC	CCGCACCGCT

Figure 26T

19001	AGCCTCCCTC	GTACGAGGAG	GCACTAAAGC	AAGGCCTGCC	CACCACCCGT
13001	TCGGAGGGAG	CATGCTCCTC	CGTGATTTCG	TTCCGGACGG	GTGGTGGGCA
19051	CCCATCGCGC	CCATGGCTAC	CGGAGTGCTG	GGCCAGCACA	CACCCGTAAC
	GGGTAGCGCG	GGTACCGATG	GCCTCACGAC	CCGGTCGTGT	GTGGGCATTG
					CMCCMCCCAC
19101	GCTGGACCTG	CCTCCCCCG	CCGACACCCA	GCAGAAACCT	CACCACCCAC
	CGACCTGGAC	GGAGGGGGGC	GGCTGTGGGT	CGTCTTTGGA	CACGACGGIC
	0000070000	CCTTCTTCTA	ACCCGTCCTA	GCCGCGCGTC	CCTGCGCCGC
19151	CCCCACCCC	GCAACAACAT	TGGGCAGGAT	CGGCGCGCAG	GGACGCGGCG
	0000010000	00,210121211			
19201	GCCGCCAGCG	GTCCGCGATC	GTTGCGGCCC	GTAGCCAGTG	GCAACTGGCA
	CGGCGGTCGC	CAGGCGCTAG	CAACGCCGGG	CATCGGTCAC	CGTTGACCGT
19251	AAGCACACTG	AACAGCATCG	TGGGTCTGGG	GGTGCAATCC	CTGAAGCGCC
	TTCGTGTGAC	TTGTCGTAGC	ACCCAGACCC	CCACGTTAGG	GACTICGCGG
		G=01=10001	******************	TGTGTGTCAT	GTATGCGTCC
19301	GACGATGCTT	CTGATAGCTA	TCC>C>CCAT	ACACACAGTA	CATACGCAGG
	CTGCTACGAA	GACTATCGAT	IGCACAGCAI	Meirerie	
19351	אייכיירפררפר	САСАССАССТ	GCTGAGCCGC	CGCGCGCCCG	CTTTCCAAGA
19351	TACAGOGGGG	GTCTCCTCGA	CGACTCGGCG	ecececeec	GAAAGGTTCT
19401	TGGCTACCCC	TTCGATGATG	CCGCAGTGGT	CTTACATGCA	CATCTCGGGC
_, _,	ACCGATGGGG	AAGCTACTAC	GGCGTCACCA	GAATGTACGT	GTAGAGCCCG
					mm00000000
19451	CAGGACGCCT	CGGAGTACCT	GAGCCCCGGG	CTGGTGCAGT	TIGUCUGUGU
	GTCCTGCGGA	GCCTCATGGA	CTCGGGGCCC	GACCACGTCA	AACGGGCGCG
		m> cmmc> ccc	mc>>m>>	GTTTAGAAAC	CCCACGGTGG
19501	CACCGAGACG	ATCANCTCGG	ACTTATTCTT	CAAATCTTTG	GGGTGCCACC
	GIGGCICIGC	AIGAAGICGG	ACTIMITO		
19551	CCCCTACGCA	CGACGTGACC	ACAGACCGGT	CCCAGCGTTT	GACGCTGCGG
17771	GCGGATGCGT	GCTGCACTGG	TGTCTGGCCA	GGGTCGCAAA	CTGCGACGCC
19601	TTCATCCCTG	TGGACCGTGA	GGATACTGCG	TACTCGTACA	AGGCGCGGTT
	AAGTAGGGAC	ACCTGGCACT	CCTATGACGC	ATGAGCATGT	TCCGCGCCAA
19651	CACCCTAGCT	GTGGGTGATA	ACCGTGTGCT	GGACATGGCT	ACCTCCATCA
	GTGGGATCGA	CACCCACTAT	TGGCACACGA	CCIGIACCOA	AGGTGCATGA
		CCCCCTCCTC	CACAGGGGC	CTACTTTAA	GCCCTACTCT
19701	TIGACATCCG	CCCCACGAC	CTGTCCCCGG	GATGAAAATT	CGGGATGAGA
	AACIGIAGGC	GCCGCACCAC	0.0.0.0		
19751	CCCACTCCCT	ACAACGCCCT	GGCTCCCAAG	GGTGCCCCAA	ATCCTTGCGA
47/34	CCGTGACGGA	TGTTGCGGGA	CCGAGGGTTC	CCACGGGGTT	TAGGAACGCT
19801	ATGGGATGAA	GCTGCTACTG	CTCTTGAAAT	AAACCTAGAA	GAAGAGGACG
	TACCCTACTT	CGACGATGAC	GAGAACTTT	A TTTGGATCTT	CTTCTCCTGC
		_			
19851	ATGACAACGA	AGACGAAGTA	GACGAGCAA	CTGAGCAGCA	AAAAACTCAC
	TACTGTTGCT	TCTGCTTCAT	CTGCTCGTT	GACTUGTUGT	TTTTTGAGTG

Figure 26 U

19951	TCAAATAGGT	GTCGAAGGTC	AAACACCTAA	ATATGCCGAT	AAAACATTTC
	AGTTTATCCA	CAGCTTCCAG	TTTGTGGATT	TATACGGCTA	TTTTGTAAAG
20001	AACCTGAACC	TCAAATAGGA	GAATCTCAGT	GGTACGAAAC	AGAAATTAAT
20053			CTTAGAGTCA AAAAAAGACT		
20051	GTACGTCGAC	CCTCTCAGGA	TTTTTTCTGA	TGGGGTTACT	TTGGTACAAT
20101	CGGTTCATAT	GCAAAACCCA	CAAATGAAAA	TGGAGGGCAA	GGCATTCTTG
	GCCAAGTATA	CGTTTTGGGT	GTTTACTTTT	ACCTCCCGTT	CCGTA-GAAC
20151	TAAAGCAACA	AAATGGAAAG	CTAGAAAGTC	AAGTGGAAAT	GCAATTTTTC
	ATTTCGTTGT	TTTACCTTTC	GATCTTTCAG	TTCACCTTTA	CGTTAAAAAG
20201	TCAACTACTG	AGGCAGCCGC	AGGCAATGGT	GATAACTTGA	CTCCTAAAGT
			TCCGTTACCA		
20251	GGTATTGTAC CCATAACATG	AGTGAAGATG TCACTTCTAC	TAGATATAGA ATCTATATCT	TTGGGGTCTG	TGAGTATAAA
20301	CTTACATGCC	CACTATTAAG	GAAGGTAACT	CACGAGAACT	AATGGGCCAA
	GAATGTACGG	GTGATAATTC	CTTCCATTGA	GTGCTCTTGA	TTACCCGGTT
20351	CAATCTATGC	CCAACAGGCC	TAATTACATT	GCTTTTAGGG	ACAATTTTAT
	GTTAGATACG	GGTTGTCCGG	ATTAATGTAA	CGAAAATCCC	TGTTAAAATA
20401	TGGTCTAATG	TATTACAACA	GCACGGGTAA	TATGGGTGTT	CTGGCGGGCC
	ACCAGATTAC	ATAATGTTGT	CGTGCCCATT	ATACCCACAA	GACCGCCCGG
20451	AAGCATCGCA	GTTGAATGCT	GTTGTAGATT	TGCAAGACAG	AAACACAGAG
	TTCGTAGCGT	CAACTTACGA	CAACATCTAA	ACGTTCTGTC	TTTGTGTCTC
20501	CTTTCATACC	AGCTTTTGCT	TGATTCCATT	GGTGATAGAA	CCAGGTACTT
	GAAAGTATGG	TCGAAAACGA	ACTAAGGTAA	CCACTATCTT	GGTCCATGAA
20551	TTCTATGTGG	AATCAGGCTG	TTGACAGCTA	TGATCCAGAT	GTTAGAATTA
	AAGATACACC	TTAGTCCGAC	AACTGTCGAT	ACTAGGTCTA	CAATCTTAAT
20601	TTGAAAATCA	TGGAACTGAA	GATGAACTTC	CAAATTACTG	CTTTCCACTG
	AACTTTTAGT	ACCTTGACTT	CTACTTGAAG	GTTTAATGAC	GAAAGGTGAC
20651	GGÄGGTGTGA	TTAATACAGA	GACTCTTACC	AAGGTAAAAC	CTAAAACAGG
	CCTCCACACT	AATTATGTCT	CTGAGAATGG	TTCCATTTTG	GATTTTGTCC
20701	TCAGGAAAAT	GGATGGGAAA	AAGATGCTAC	AGAATTTTCA	GATAAAAATG
	AGTCCTTTTA	CCTACCCTTT	TTCTACGATG	TCTTAAAAGT	CTATTTTTAC
20751	AAATAAGAGT	TGGAAATAAT	TTTGCCATGG	AAATCAATCT	AAATGCCAAC
	TTTATTCTCA	ACCTTTATTA	AAACGGTACC	TTTAGTTAGA	TTTACGGTTG
20801	CTGTGGAGAA	ATTTCCTGTA	CTCCAACATA	GCGCTGTATT	TGCCCGACAA
	GACACCTCTT	TAAAGGACAT	GAGGTTGTAT	CGCGACATAA	ACGGGCTGTT

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20901	ACGACTACAT TGCTGATGTA	GAACAAGCGA CTTGTTCGCT	GTGGTGGCTC CACCACCGAG	CCGGGCTAGT GGCCCGATCA	GGACTGCTAC CCTGACGATG
20951	ATTAACCTTG TAATTGGAAC	GAGCACGCTG CTCGTGCGAC	GTCCCTTGAC CAGGGAACTG	TATATGGACA ATATACCTGT	ACGTCAACCC TGCAGTTGGG
21001	ATTTAACCAC TAAATTGGTG	CACCGCAATG GTGGCGTTAC	CTGGCCTGCG GACCGGACGC	CTACCGCTCA GATGGCGAGT	ATGTTGCTGG TACAACGACC
21051	GCAATGGTCG CGTTACCAGC	CTATGTGCCC GATACACGGG	TTCCACATCC AAGGTGTAGG	AGGTGCCTCA TCCACGGAGT	GAAGTTCTTT CTTCAAGAAA
21101	GCCATTAAAA CGGTAATTTT	ACCTCCTTCT TGGAGGAAGA	CCTGCCGGGC	TCATACACCT AGTATGTGGA	ACGAGTGGAA TGCTCACCTT
21151	GAAGTCCTTC	CTACAATTGT	TGGTTCTGCA ACCAAGACGT	CTCGAGGGAT	CCTTTACTGG
21201	ATTCCCAACT	GCCTCGGTCG	ATTAAGTTTG TAATTCAAAC	TATCGTAAAC	GGAAATGCGG
21251	TGGAAGAAGG	GGTACCGGGT	CAACACCGCC GTTGTGGCGG	AGGTGCGAAC	TCCGGTACGA
21301	ATCTTTGCTG	TGGTTGCTGG	AGTCCTTTAA TCAGGAAATT	GCTGATAGAG	AGGCGGCGGT
21351	TGTACGAGAT	GGGATATGGG	GCCAACGCTA CGGTTGCGAT	GGTTGCACGG	GTATAGGTAG
21401	GGGAGGGCGT	TGACCCGCCG	TTTCCGCGGC AAAGGCGCCG	ACCCGGAAGT	GCGCGGAATT
21451	CTGATTCCTT	TGGGGTAGTG	TGGGCTCGGG ACCCGAGCCC	GATGCTGGGA	ATAATGTGGA
2,1501	TGAGACCGAG	ATATGGGATG	CTAGATGGAA GATCTACCTT	ggaaaatgga	GTTGGTGTGG
21551	AAATTCTTCC	ACCGGTAATG	GAAACTGAGA	AGACAGTCGA	GGCCTGGCAA
	ACTGGCGGAC	GAATGGGGGT	TGCTCAAACT	TTAATTCGCG	TCAGTTGACG AGTCAACTGC
	CCCTCCCAAT	GTTGCAACGG	GTCACATTGT	ACTGGTTTCT	CTGGTTCCTG
	CATGTTTACG	ATCGATTGAT	ATTGTAACCG	ATGGTCCCGA	TCTATATCCC AGATATAGGG
21751	AGAGAGCTAC TCTCTCGATG	AAGGACCGCA TTCCTGGCGT	TGTACTCCTT	CTTTAGAAAC GAAATCTTTG	TTCCAGCCCA AAGGTCGGGT

Figure 26 W

21851	GGCATCCTAC	ACCAACACAA	CAACTCTGGA	TTTGTTGGCT	ACCTTGCCCC
	CCGTAGGATG	TGGTTGTGTT	GTTGAGACCT	AAACAACCGA	TGGAACGGGG
21901				TAACTTCCCC ATTGAAGGGG	
21951	TAGGCAAGAC	CGCAGTTGAC	AGCATTACCC	AGAAAAAGTT	TCTTTGCGAT
	ATCCGTTCTG	GCGTCAACTG	TCGTAATGGG	TCTTTTTCAA	AGAAACGCTA
22001	CGCACCCTTT	GGCGCATCCC	ATTCTCCAGT	AACTTTATGT	CCATGGGCGC
	GCGTGGGAAA	CCGCGTAGGG	TAAGAGGTCA	TTGAAATACA	GGTACCCGCG
22051	ACTCACAGAC	CTGGGCCAAA	ACCTTCTCTA	CGCCAACTCC	GCCCACGCGC
	TGAGTGTCTG	GACCCGGTTT	TGGAAGAGAT	GCGGTTGAGG	CGGGTGCGCG
22101	TAGACATGAC ATCTGTACTG	TTTTGAGGTG AAAACTCCAC	GATCCCATGG CTAGGGTACC	ACGAGCCCAC TGCTCGGGTG	CCTTCTTTAT GGAAGAAATA
22151	GTTTTGTTTG	AAGTCTTTGA	CGTGGTCCGT	GTGCACCAGC	CGCACCGCGG
	CAAAACAAAC	TTCAGAAACT	GCACCAGGCA	CACGTGGTCG	GCGTGGCGCC
22201	CGTCATCGAA	ACCGTGTACC	TGCGCACGCC	CTTCTCGGCC	GGCAACGCCA
	GCAGTAGCTT	TGGCACATGG	ACGCGTGCGG	GAAGAGCCGG	CCGTTGCGGT
22251	CAACATAAAG	AAGCAAGCAA	CATCAACAAC	AGCTGCCGCC	ATGGGCTCCA
	GTTGTATTTC	TTCGTTCGTT	GTAGTTGTTG	TCGACGGCGG	TACCCGAGGT
22301	GTGAGCAGGA	ACTGAAAGCC	ATTGTCAAAG	ATCTTGGTTG	TGGGCCATAT
	CACTCGTCCT	TGACTTTCGG	TAACAGTTTC	TAGAACCAAC	ACCCGGTATA
22351	TTTTTGGGCA	CCTATGACAA	GCGCTTTCCA	GGCTTTGTTT	CTCCACACAA
	AAAAACCCGT	GGATACTGTT	CGCGAAAGGT	CCGAAACAAA	GAGGTGTGTT
22401				TCGCGAGACT AGCGCTCTGA	
22451	ACTGGATGGC	CTTTGCCTGG	AACCCGCACT	CAAAAACATG	CTACCTCTTT
	TGACCTACCG	GAAACGGACC	TTGGGCGTGA	GTTTTTGTAC	GATGGAGAAA
22501	GAGCCCTTTG	GCTTTTCTGA	CCAGCGACTC	AAGCAGGTTT	ACCAGTTTGA
	CTCGGGAAAC	CGAAAAGACT	GGTCGCTGAG	TTCGTCCAAA	TGGTCAAACT
		GAGGACGCGG	CATCGCGGTA	ACGAAGAAGG	GGGCTGGCGA
22601	GTATAACGCT	GGAAAAGTCC	ACCCAAAGCG	TACAGGGGCC	CAACTCGGCC
	CATATTGCGA	CCTTTTCAGG	TGGGTTTCGC	ATGTCCCCGG	GTTGAGCCGG
22651	GCCTGTGGAC	TATTCTGCTG	CATGTTTCTC	CACGCCTTTG	CCAACTGGCC
	CGGACACCTG	ATAAGACGAC	GTACAAAGAG	GTGCGGAAAC	GGTTGACCGG
22701	CCAAACTCCC	ATGGATCACA	ACCCCACCAT	GAACCTTATT	ACCGGGGTAC
	GGTTTGAGGG	TACCTAGTGT	TGGGGTGGTA	CTTGGAATAA	TGGCCCCATG

Figure 26 X

•	<i>J UZ/UZZU</i>	5 U				101/001
	22801	CAGGAACAGC GTCCTTGTCG	TCTACAGCTT AGATGTCGAA	CCTGGAGUGU GGACCTCGCG	CACTCGCCC1 GTGAGCGGGA	TGAAGGCGTC
	22851	CCACAGTGCG	CAGATTAGGA GTCTAATCCT	GCGCCACTTC CGCGGTGAAG	TTTTTGTCAC AAAAACAGTG	TTGAAAAACA AACTTTTTGT
	22901	መርመል ል ል መጋመ	ATGTACTAGA TACATGATCT	GACACTTTCA	ATAAAGGCAA	ATGCTTTTAT
			TCGGGTGATT			
	22951	AACATGTGAG	AGCCCACTAA	TAAATGGGGG	TGGGAACGGC	AGACGCGGCA
	23001	TTAAAAATCA AATTTTTAGT	AAGGGGTTCT TTCCCCAAGA	GCCGCGCATC CGGCGCGTAG	GCTATGCGCC CGATACGCGG	TGACCGTCCC
	23051	ACACGTTGCG TGTGCAACGC	ATACTGGTGT TATGACCACA	TTAGTGCTCC AATCACGAGG	ACTTAAACTC TGAATTTGAG	AGGCACAACC TCCGTGTTGG
	23101	ATCCGCGGCA TAGGCGCCGT	GCTCGGTGAA CGAGCCACTT	GTTTTCACTC CAAAAGTGAG	CACAGGCTGC GTGTCCGACG	GCACCATCAC CGTGGTAGTG
	23151	C	AGCAGGTCGG TCGTCCAGCC	GCGCCGATAT	CTTGAAGTCG	CAGTTGGGGC
	23201	C#CCCCCTG	CGCGCGCGAG GCGCGCGCTC	TTGCGATACA	CAGGGTTGCA	GCACTGGAAC
	23251	NCT:NTCNCCC	ССССТССТС	CACGCTGGCC	AGCACGCTCT	TGTCGGAGAT
	23301	0.00mccccc	GGCCCACCAC	CCGCGTTGCT	CAGGGCGAAC	GGAGTCAACT
		GTCTAGGCGC	AGGTCCAGGA	GGCGCAACGA	, GTCCCGCTTG	CCTCAGTTGA
	23351	AACCATCGAC	GGAAGGGTTT	TTCCCGCGCA	. CGGGTCCGAF	ACTUAACGIG
	23401	AGCGTGGCAT	CACCGTAGTT	TTCCACTGGC	: ACGGGCCAGA	GGGCGTTAGG CCCGCAATCC
	23451	ATACAGCGCC TATGTCGCGC	TGCATAAAAG ACGTATTTTC	CCTTGATCTC GGAACTAGAC	GAATTTTCG	ACCTGAGCCT G TGGACTCGGA
	23501	TTGCGCCTT(AGAGAAGAAC TOTOTTOTTC	TACGGCGTT	ACTTGCCGG	A AAACTGATTG TTTGACTAAC
	23551	GCCGGACAG(CCGCGTCGTC	CACGCAGCA(CAC	CTTGCGTCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	G TGTTGGAGAT C ACAACCTCTA
	23601	CTGCACCAC	A TTTCGGCCCC	ACCGGTTCT	T CACGATCTT A GTGCTAGAA	G GCCTTGCTAG C CGGAACGATC
	23651	*	T CAGCGCGCG	TGCCCGTTT	T CGCTCGTCA	C ATCCATTTCA G TAGGTAAAGT
		TGACGAGGA	W Gicacacac			

Figure 26 Y

WO 02/022080					PCT/US01/28861
23701	ATCACGTGCT	CCTTATTTAT	CATAATGCTI	CCGTGTAGAC	ACTTAAGCTC
				GGCACATCTG	
23751	GCCTTCGATC	TCAGCGCAGC	GGTGCAGCCA	CAACGCGCAG	CCCGTGGGCT
	CGGAAGCTAG	AGTCGCGTCG	CCACGTCGGT	GTTGCGCGTC	GGGCACCCGA
23801			• •	ACTGCAGGTA	
	GCACTACGAA	CATCCAGTGG	AGACGTTTGC	TGACGTCCAT	GCGGACGTCC
23851				TTGCTGGTGA	
	TTAGCGGGGT	AGTAGCAGTG	TTTCCAGAAC	AACGACCACT	TCCAGTCGAC
23901	CAACCCGCGG	TGCTCCTCGT	TCAGCCAGGT	CTTGCATACG	GCCGCCAGAG
	GTTGGGCGCC	ACGAGGAGCA	AGTCGGTCCA	GAACGTATGC	CGGCGGTCTC
23951	CTTCCACTTG	GTCAGGCAGT	AGTTTGAAGT	TCGCCTTTAG	ATCGTTATCC
	GAAGGTGAAC	CAGTCCGTCA	TCAAACTTCA	AGCGGAAATC	TAGCAATAGG
24001	ACGTGGTACT	TGTCCATCAG	CGCGCGCGCA	GCCTCCATGC	CCTTCTCCCA
				CGGAGGTACG	
24051				CATCACCGTA	
				GTAGTGGCAT	
24101				GCGTCCGCAT	
				CGCAGGCGTA	
24151				GTGCGCTTAC	
				CACGCGAATG	
24201	-			ACCCACCATT	
0.4054				TGGGTGGTAA	•
24251				TTACCTCTGG AATGGAGACC	
24301				TTCTTCTTGG	
24501				AAGAAGAACC	
24251				GCTGGGTGTG	
24331				CGACCCACAC	
•					
				CGGACTCGAT	
				GCCTGAGCTA	
24451				GGCGGCGACG	
				CCGCCGCTGC	
24501				CGCCGCACCG	
				GCGGCGTGGC	
24551				GACTGGCCAT	
				CTGACCGGTA	•
24601				GAGAAGAAGG	
	ATATCCGTCT	TTTTCTAGTA	CCTCAGTCAG	CTCTTCTTCC	TGTCGGATTG

Figure 262

24701	CTACCACCTT	CCCCGTCGAG	GCACCCCCGC	TTGAGGAGGA	GGAAGTGATT
24/01	CATGGTGGAA	GGGGCAGCTC	CGTGGGGGCG	AACTCCTCCT	CCTTCACTAA
24751	ATCGAGCAGG	ACCCAGGTTT	TGTAAGCGAA	GACGACGAGG	ACCGCTCAGT
24/54	TAGCTCGTCC	TGGGTCCAAA	ACATTCGCTT	CTGCTGCTCC	TGGCGAGTCA
24801	ACCAACAGAG	GATAAAAAGC	AAGACCAGGA	CAACGCAGAG	GCAAACGAGG
24001	TGGTTGTCTC	CTATTTTTCG	TTCTGGTCCT	GTTGCGTCTC	CGTTTGCTCC
		•			
24851	AACAAGTCGG	GCGGGGGGAC	GAAAGGCATG	GCGACTACCT	agatgtggga
	TTGTTCAGCC	CGCCCCCTG	CTTTCCGTAC	CGCTGATGGA	TCTACACCCT
24901	GACGACGTGC	TGTTGAAGCA	TCTGCAGCGC	CAGTGCGCCA	TTATCTGCGA
	CTGCTGCACG	ACAACTTCGT	AGACGTCGCG	GTCACGCGGT	AATAGACGCT
24951	CGCGTTGCAA	GAGCGCAGCG	ATGTGCCCCT	CGCCATAGCG	GATGTCAGCC
	GCGCAACGTT	CTCGCGTCGC	TACACGGGGA	GCGGTATCGC	CTACAGTCGG
25001	TTGCCTACGA	ACGCCACCTA	TTCTCACCGC	GCGTACCCCC	CAAACGCCAA
	AACGGATGCT	TGCGGTGGAT	AAGAGTGGCG	CGCATGGGGG	GTTTGCGGTT
					* 000000m* mm
25051	GAAAACGGCA	CATGCGAGCC	CAACCCGCGC	CTCAACTICT	MCCCCG1W11
	CTTTTGCCGT	GTACGCTCGG	GTTGGGCGCG	GAGTTGAAGA	TGGGGCATAA
					CANADCTCCA
25101	TGCCGTGCCA	GAGGTGCTTG	CCACCTATCA	CATCTITIC	CAMACIGCA
	ACGGCACGGT	CTCCACGAAC	GGTGGATAGT	GTAGAAAAAG	GIIIIGACOX
				CCCCACCGGA	CAAGCAGCTG
25151	AGATACCCCT	TAGGACGGCA	CCCMACCCCA	CCCCTCCCCT	GTTCGTCGAC
	TCTATGGGGA	TAGGACGGCA	(661166661	0000100001	0
			ことがなっているので	**TCGCCTCGC	TCAACGAAGT
25201	GCCTTGCGGC	AGGGCGC1G1	CTATCCACTA	TAGCGGAGCG	AGTTGCTTCA
	CGGAACGCCG	TECEGEGACA	GIAI GGACI		
05051	CCC	· 'ጥጥጥር አርርርጥር	TTGGACGCGA	CGAGAAGCGC	GCGGCAAACG
25251	GCCAAAAA1C	. Illumouce.c	AACCTGCGCT	GCTCTTCGCG	CGCCGTTTGC
	CGGIIIIAG	MANCICCCAO			
25301	CTCTCCAACA	CCAAACACC	GAAAATGAAA	GTCACTCTGG	AGTGTTGGTG
25301	CACACCTTCT	CCTTTTGTCG	CTTTTACTT	CAGTGAGACC	TCACAACCAC
25351	CAACTCGAGG	GTGACAACGC	GCGCCTAGCC	GTACTAAAAC	GCAGCATCGA
23331	CTTGAGCTCC	CACTGTTGCG	CGCGGATCGG	CATGATTTTC	CGTCGTAGCT
25401	GGTCACCCAC	TTTGCCTACC	CGGCACTTAA	CCTACCCCC	AAGGTCATGA
23402	CCAGTGGGT	AAACGGATGG	GCCGTGAATT	GGATGGGGG	TTCCAGTACT
	,				
25451	GCACAGTCAT	CAGTGAGCTG	ATCGTGCGCC	GTGCGCAGC	CCTGGAGAGG
25,100	CGTGTCAGTA	A CTCACTCGAC	TAGCACGCGG	CACGCGTCGC	GGACCTCTCC
25501	GATGCAAAT'	T TGCAAGAACA	AACAGAGGAG	GCCTACCC	CAGTTGGCGA
	CTACGTTTA	A ACGTTCTTGT	TTGTCTCCT(CCGGATGGG	GTCAACCGCT
25551	CGAGCAGCT	A GCGCGCTGG	TTCAAACGC	CGAGCCTGC	GACTTGGAGG
	GCTCGTCGA'	T CGCGCGACCC	AAGTTTGCG	C GCTCGGACG	CTGAACCTCC

7 igure 26 AA

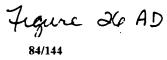
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25701				CGTACGCCAG GCATGCGGTC	
25751				CCTACCTTGG GGATGGAACC	
25801				TCCACGCTCA AGGTGCGAGT	
25851	GCGCCGCGAC CGCGGCGCTG	TACGTCCGCG ATGCAGGCGC	ACTGCGTTTA TGACGCAAAT	CTTATTTCTA GAATAAAGAT	TGCTACACCT ACGATGTGGA
25901				GCTTGGAGGA CGAACCTCCT	
25951				TTGAAGGACC AACTTCCTGG	
26001				GGCGGACATC CCGCCTGTAG	
26051				TGCCAGACTT ACGGTCTGAA	
26101				CTAGAGCGCT GATCTCGCGA	
26151				CTTTGTGCCC GAAACACGGG	
26201				GCTACCTTCT CGATGGAAGA	
26251	TTGATGGAAC	GGATGGTGAG	ACTGTATTAC	GAAGACGTGA CTTCTGCACT	CGCCACTGCC
26301	AGATGACCTC	ACAGTGACAG	CGACGTTGGA		GTGGCGAGGG
	TGGTTTGCAA ACCAAACGTT	AAGCGTCGAC	GAATTGCTTT	CAGTTTAATA	GCCATGGAAA
	GAGCTGCAGG CTCGACGTCC	CAGGGAGCGG	ACTGCTTTTC	AGGCGCCGAG	GCCCCAACTT
26451	ACTCACTCCG TGAGTGAGGC	GGGCTGTGGA CCCGACACCT	CGTCGGCTTA GCAGCCGAAT	CCTTCGCAAA GGAAGCGTTT	TTTGTACCTG AAACATGGAC
26501	AGGACTACCA TCCTGATGGT	CGCCCACGAG GCGGGTGCTC	ATTAGGTTCT TAATCCAAGA	ACGAAGACCA TGCTTCTGGT	ATCCCGCCCG TAGGGCGGGC

Figure 26 AB 82/144

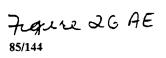
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26551	GGATTACGCC	TCGAATGGCG	GACGCAGTAA	TGGGTCCCGG	TGTAAGAACC
26601	CC > > TTTCC > >	CCCATCAACA	AAGCCCGCCA	AGAGTTTCTG	CTACGAAAGG
26601	GGTTAACGTT	CGGTAGTTGT	TTCGGGCGGT	TCTCAAAGAC	GATGCTTTCC
26651	GACGGGGGGT	TTACTTGGAC	CCCCAGTCCG	GCGAGGAGCT	CAACCCAATC
	CTGCCCCCCA	AATGAACCTG	GGGGTCAGGC	CGCTCCTCGA	GTTGGGTTAG
26701	CCCCGCCGC	CGCAGCCCTA	TCAGCAGCAG	CCCCGGGCCC	TTGCTTCCCA
			AGTCGTCGTC		
26751	GGATGGCACC	CAAAAAGAAG	CTGCAGCTGC	CGCCGCCACC	CACGGACGAG
	CCTACCGTGG	GTTTTTCTTC	GACGTCGACG	GCGGCGGTGG	GTGCCTGCTC
26801	GAGGAATACT	GGGACAGTCA	GGCAGAGGAG	GTTTTGGACG	AGGAGGAGGA
	CTCCTTATGA	CCCTGTCAGT	CCGTCTCCTC	CAAAACCTGC	TCCTCCTCCT
26851	GGACATGATG	GAAGACTGGG	AGAGCCTAGA	CGAGGAAGCT	TCCGAGGTCG
			TCTCGGATCT		
26901	AAGAGGTGTC	AGACGAAACA	CCGTCACCCT	CGGTCGCATT	CCCCTCGCCG
			GGCAGTGGGA		
26951	GCGCCCCAGA	AATCGGCAAC	CGGTTCCAGC	ATGGCTACAA	CCACCCCACC
			GCCAAGGTCG		
27001	TCAGGCGCCG	CCGGCACTGC	CCGTTCGCCG	MCCCMACCG1	TOTACCOTOT
			GGCAAGCGGC		
27051	CCACTGGAAC	CAGGGCCGGT	AAGTCCAAGC	TCCCCCCCCC	CAATCGGGTT
			TTCAGGTTCG		
27101	GAGCAACAAC	AGCGCCAAGG	CTACCGCTCA GATGGCGAGT	ACCCCCCCCCC	ተርተተርተተርርG
	-		ACTGTGGGGG		
27151	CATAGTIGCT	NCC NCCTTC	TGACACCCCC	GTTGTAGAGG	AAGCGGCCGG
07001					CATCCTGCAT
27201	GCTTTCTTCT	CIACCATCAC	CCGCACCGGA	AGGGGGCATT	GTAGGACGTA
	-				
27251	TACTACCGTC	ATCTCTACAG	CCCATACTGC	ACCGGCGGCA	GCGGCAGCAA
	ATGATGGCAG	TAGAGATGTC	: GGGTATGACG	TGGCCGCCGT	CGCCGTCGTT
27301	CAGCAGCGGC	CACACAGAAG	CAAAGGCGAC	CGGATAGCAA	GACTCTGACA
	GTCGTCGCCG	GTGTGTCTTC	: GTTTCCGCTG	GCCTATCGTI	CTGAGACTGT
27351	AAGCCCAAGA	AATCCACAGO	GGCGGCAGCA	GCAGGAGGAC	GAGCGCTGCG
	TTCGGGTTCT	TTAGGTGTC	CCGCCGTCG1	CGTCCTCCTC	CTCGCGACGC
27401	TCTGGCGCCC	AACGAACCC	TATCGACCCG	CGAGCTTAG	AACAGGATTT
	AGACCGCGGG	TTGCTTGGG	ATAGCTGGGC	GCTCGAATCT	TTGTCCTAAA
27451	TTCCCACTCT	GTATGCTAT	A TTTCAACAGA	A GCAGGGGCC	AGAACAAGAG
	AAGGGTGAGA	CATACGATA	r AAAGTTGTC	r cgrccccgg:	TCTTGTTCTC

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27551	TCACAAAAGC	GAAGATCAGC	TTCGGCGCAC	GCTGGAAGAC	GCGGAGGCTC
			AAGCCGCGTG		
27601	TCTTCAGTAA	ATACTGCGCG	CTGACTCTTA	AGGACTAGTT	TCGCGCCCTT
	AGAAGTCATT	TATGACGCGC	GACTGAGAAT	TCCTGATCAA	AGCGCGGGAA
27651	TCTCAAATTT	AAGCGCGAAA	ACTACGTCAT	CTCCAGCGGC	CACACCCGGC
	AGAGTTTAAA	TTCGCGCTTT	TGATGCAGTA	GAGGTCGCCG	GTGTGGGCCG
27701	GCCAGCACCT	GTTGTCAGCG	CCATTATGAG	CAAGGAAATT	CCCACGCCCT
			GGTAATACTC		
27751	ACATGTGGAG	TTACCAGCCA	CAAATGGGAC	TTGCGGCTGG	AGCTGCCCAA
			GTTTACCCTG		
27801	GACTACTCAA	CCCGAATAAA	CTACATGAGC	GCGGGACCCC	ACATGATATC
			GATGTACTCG		
27851	CCGGGTCAAC	GGAATACGCG	CCCACCGAAA	CCGAATTCTC	CTGGAACAGG
			GGGTGGCTTT		
27901	CGGCTATTAC	CACCACACCT	CGTAATAACC	TTAATCCCCG	TAGTTGGCCC
			GCATTATTGG		
27951	GCTGCCCTGG	TGTACCAGGA	AAGTCCCGCT	CCCACCACTG	TGGTACTTCC
	•		TTCAGGGCGA		
28001-	CAGAGACGCC	CAGGCCGAAG	TTCAGATGAC	TAACTCAGGG	GCGCAGCTTG
			AAGTCTACTG		
28051	CGGGCGCTT	TCGTCACAGG	GTGCGGTCGC	CCGGGCAGGG	TATAACTCAC
			CACGCCAGCG		•
28101	CTGACAATCA	GAGGGCGAGG	TATTCAGCTC	AACGACGAGT	CGGTGAGCTC
			ATAAGTCGAG		
28151	CTCGCTTGGT	CTCCGTCCGG	ACGGGACATT	TCAGATCGGC	GGCGCCGGCC
			TGCCCTGTAA		
28201	GCTCTTCATT	CACGCCTCGT	CAGGCAATCC	TAACTCTGCA	GACCTCGTCC
					CTGGAGCAGG
28251	TCTGAGCCGC	GCTCTGGAGG	CATTGGAACT	CTGCAATTTA	TTGAGGAGTT
					AACTCCTCAA
28301	TGTGCCATCG	GTCTACTTTA	ACCCCTTCTC	GGGACCTCCC	GGCCACTATC
					CCGGTGATAG
28351	CGGATCAATT	TATTCCTAAC	TTTGACGCGG	TAAAGGACTC	GGCGGACGGC
					CCGCCTGCCG
28401	TACGACTGAA	TGTTAAGTGG	AGAGGCAGAG	CAACTGCGCC	TGAAACACCT
	ATGCTGACTT	ACAATTCACC	TCTCCGTCTC	GTTGACGCGG	ACTTTGTGGA



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• • • • • • • •					
28451	GGTCCACTGT (CCAGGTGACA (CGCCGCCACA : CCGCCGGTGT :	AGTGCTTTGC ICACGAAACG	GGCGCTGAGG	CCACTCAAAA
					0010000000
28501	GCTACTTTGA Z	ATTGCCCGAG(TAACGGGCTC(GATCATATCG CTAGTATAGC	TCCCGGGCCGGC	CGTGCCGCAG
28551	CGGCTTACCG	CCCAGGGAGA (GCTTGCCCGT	AGCCTGATTC	GGGAGTTTAC
	GCCGAATGGC				
28601	CCAGCGCCCC	CTGCTAGTTG	AGCGGGACAG	GGGACCCTGT	GTTCTCACTG
20002	GGTCGCGGGG	GACGATCAAC (TCGCCCTGTC	CCCTGGGACA	CAAGAGTGAC
					መመርመ ምርርር አጥ
28651	TGATTTGCAA	CTGTCCTAAC	CCTGGATTAC	ATCAAGATCT	ANCANCECTA
	ACTAAACGTT				
28701	CTCTGTGCTG	AGTATAATAA	ATACAGAAAT	TAAAATATAC	TGGGGCTCCT
20.02	GAGACACGAC	TCATATTATT	TATGTCTTTA	ATTTTATATG	ACCCCGAGGA
				00000000330	CARACCARG
28751	ATCGCCATCC	TGTAAACGCC	ACCGTCTTCA	CCCGCCCAAG	CAMACCAAGG
	TAGCGGTAGG				
28801	CGAACCTTAC	CTGGTACTTT	TAACATCTCT	CCCTCTGTGA	TTTACAACAG
20001	GCTTGGAATG	GACCATGAAA	ATTGTAGAGA	GGGAGACACT	AAATGTTGTC
20051	TTTCAACCCA	CACGGAGTGA	GTCTACGAGA	GAACCTCTCC	GAGCTCAGCT
. 28821	AAAGTTGGGT	CTGCCTCACT	CAGATGCTCT	CTTGGAGAGG	CTCGAGTCGA
				CCTCCCCCA	ACGTACGAGT
28901	ACTCCATCAG	AAAAAACACC	TGGGAGGAAT	TOOODOO	TGCATGCTCA
28951	CCCTCACCGG	CCGCTGCACC	ACACCTACCG	CCTGACCGTA	AACCAGACTT
20931	CGCAGTGGCC	GGCGACGTGG	TGTGGATGGC	GGACTGGCAT	TTGGTCTGAA
20001	TTTCCGGACA	CACCTCAATA	ACTCTGTTTA	CCAGAACAGG	AGGTGAGCTT
29001	AAAGGCCTGT	CTGGAGTTAT	TGAGACAAAT	GGTCTTGTCC	TCCACTCGAA
		m><	CCCCAAAGGC	GCAGCTACTG	TGGGGTTTAT
29051	AGAAAACCCT	ATCCCATAAT	CCGGTTTCCG	CGTCGATGAC	ACCCCAAATA
29101	GAACAATTCA	AGCAACTCTA	CGGGCTATTC	TAATTCAGGI	TTCTCTAGAA
	CTTGTTAAGT	TCGTTGAGAT	GCCCGATAAG	ATTAAGTCUA	AAGAGAICII
20151	ም ር ርር ርር ርጥጥር ር	GGTTATTCTC	TGTCTTGTGA	TTCTCTTTAT	TCTTATACTA
29131	AGCCCCAACC	CCAATAAGAG	ACAGAACACT	AAGAGAAATA	AGAATATGAT
	1 = 00m=0m2m		רפררפררזיפר	TGTGTGCAC	TTTGCATTTA
29201	ACGCTTCTCT	CCCIMAGGCI	CCCCCCGACG	ACACACGTG	T AAACGTAAAT
20251	TTGTCAGCTT	TTTAAACGCT	GGGGTCGCC	CCCAAGATG	TTAGGTACAT
29231	AACAGTCGAA	AAATTTGCGA	CCCCAGCGG	GGGTTCTAC	r AATCCATGTA
	እ አጥሮርጥን ርርጥ	TTACTCACCC	TTGCGTCAG	CCACGGTAC	C ACCCAAAAGG
29301	TTAGGATCCA	AATGAGTGGG	AACGCAGTC	G GGTGCCATG	G TGGGTTTTCC
					o መርአ አርርጥላ አጥ
29351	TGGATTTTAA	GGAGCCAGCC CCTCGGTCGG	TGTAATGTTA ACATTACAA	A CATTCGCAG T GTAAGCGTC	C TGAAGCTAAT G ACTTCGATTA



29451				TGTTTATGCT ACAAATACGA	
29501				TTTTCCAGGG	
	GTCCACTGTG	ATGTCTCATA	TTACAATGTC	AAAAGGTCCC	ATTTTCAGTA
29551				GAAATGTGCG CTTTACACGC	
29601				CCCACAAAAT GGGTGTTTTA	
29651				TAATTACAGT ATTAATGTCA	
29701	GTCTGTACCC	TACTCTATAT	TAAATACAAA	AGCAGACGCA	GCTTTATTGA
				TCGTCTGCGT	
29751				ACAAAGCTAA TGTTTCGATT	
29801				TTCAAAAAGT AAGTTTTTCA	
29851	ATTAGAATAG	GATTTAAACC	CCCCGGTCAT	TTCCTGCTCA	ATACCATTCC
2,002				AAGGACGAGT	
29901				TCCAGCGCTA	
				AGGTCGCGAT	
29951				TTGGCCAGCA AACCGGTCGT	
30001	GGATTTGTTC	CAGTCCAACT	ACAGCGACCC	ACCCTAACAG	AGATGACCAA
30001				TGGGATTGTC	
30051				TACATCTACC	
				ATGTAGATGG	
30101				ATAACTTGGG TATTGAACCC	
20151	TTCTCCATAG				
30131				TAATAATACA	
30201	CTGCCTAAAG				
				GTAGATATCA	
30251	TGCTACACCC			GATTGGACGG CTAACCTGCC	
20201	ATGTTCTTTT				
30201				CTCTGTACTA	

Figure 26 AF

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30401	TGCGGTTTCT	CACATCGAAG	TAGACTGCAT	TCCAGCCTTC	ACAGTCTATT
			ATCTGACGTA		
30451	TGCTTTACGG	ATTTGTCACC	CTCACGCTCA	TCTGCAGCCT	CATCACTGTG
	ACGAAATGCC	TAAACAGTGG	GAGTGCGAGT	AGACGTCGGA	GTAGTGACAC
30501	GTCATCGCCT	TTATCCAGTG	CATTGACTGG	GTCTGTGTGC	GCTTTGCATA
			GTAACTGACC		
30551	TCTCAGACAC	CATCCCCAGT	ACAGGGACAG	GACTATAGCT	GAGCTTCTTA
			TGTCCCTGTC		
30601	GAATTCTTTA	ATTATGAAAT	TTACTGTGAC	TTTTCTGCTG	ATTATTTGCA
		•	AATGACACTG		
30651	CCCTATCTGC	GTTTTGTTCC	CCGACCTCCA	AGCCTCAAAG	ACATATATCA
			GGCTGGAGGT		
30701	TGCAGATTCA	CTCGTATATG	GAATATTCCA	AGTTGCTACA	ATGAAAAAAG
			CTTATAAGGT		
30751	CGATCTTTCC	GAAGCCTGGT	TATATGCAAT	CATCTCTGTT	ATGGTGTTCT
			ATATACGTTA		
30801	GCAGTACCAT	CTTAGCCCTA	GCTATATATC	CCTACCTTGA	CATTGGCTGG
			CGATATATAG		
30851	AACGCAATAG	ATGCCATGAA	CCACCCAACT	TTCCCCGCGC	CCGCTATGCT
			GGTGGGTTGA		
30901	TCCACTGCAA	CAAGTTGTTG	CCGGCGGCTT	TGTCCCAGCC	AATCAGCCTC
			GGCCGCCGAA		
30951	GCCCACCTTC	TCCCACCCCC	ACTGAAATCA	GCTACTTTAA	TCTAACAGGA
			TGACTTTAGT		
31001	GGAGATGACT	GACACCCTAG	ATCTAGAAAT	GGACGGAATT	ATTACAGAGC
			TAGATCTTTÀ		
31051	AGCGCCTGCT	AGAAAGACGC	AGGGCAGCGG	CCGAGCAACA	GCGCATGAAT
					CGCGTACTTA
31101	CAAGAGCTCC	AAGACATGGT	TAACTTGCAC	CAGTGCAAAA	GGGGTATCTT
					CCCCATAGAA
31151	TTGTCTCGTA	AAGCAGGCCA	AAGTCACCTA	CGACAGTAAT	ACCACCGGAC
					TGGTGGCCTG
31201	ACCGCCTTAG	CTACAAGTTG	CCAACCAAGC	GTCAGAAATT	GTGGTCATG
	TGGCGGAATO	GATGTTCAAC	: GGTTGGTTCG	CAGTCTTA	CCACCAGTAC
31251	GTGGGAGAAA	AGCCCATTAC	CATAACTCAG	CACTCGGTAC	AAACCGAAGG
	CACCCTCTTT	TCGGGTAAT	GTATTGAGTC	: GTGAGCCAT(TTTGGCTTCC

Figure 26 A6 87/144

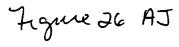
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31451		 CCCTCCTCCC	TTGCAGCTTC AACGTCGAAG
31501		 CCACAATCTA GGTGTTAGAT	
31551		 CCACTATCTT GGTGATAGAA	
31601		 ACCTTCAACC TGGAAGTTGG	
31651		 GCCTTTTCTT CGGAAAAGAA	
31701		CCCCTGGGGT GGGGACCCCA	
31751		 GGCATGCTTG CCGTACGAAC	 •
31801	•	 CAACCTTACC GTTGGAATGG	
31851		CCAAGTCAAA GGTTCAGTTT	
31901		 GAAGCCCTAA CTTCGGGATT	
31951		ACTCACCATG TGAGTGGTAC	
32001		GCATTGCCAC CGTAACGGTG	
32051	CAGAAGGAAA GTCTTCCTTT	CAAACATCAG GTTTGTAGTC	
32101	AGCAGTACCC TCGTCATGGG		
32151	TAGCTTGGGC ATCGAACCCG		
32201	TAGGACTAAA ATCCTGATTT		

Figure 26 AH

32301	AACTAAAGTT TTGATTTCAA	ACTGGAGCCT TGACCTCGGA	TGGGTTTTGA ACCCAAAACT	TTCACAAGGC AAGTGTTCCG	AATATGCAAC TTATACGTTG
32351	TTAATGTAGC AATTACATCG	AGGAGGACTA TCCTCCTGAT	AGGATTGATT TCCTAACTAA	CTCAAAACAG GAGTTTTGTC	ACGCCTTATA TGCGGAATAT
32401	CTTGATGTTA GAACTACAAT	GTTATCCGTT CAATAGGCAA	TGATGCTCAA ACTACGAGTT	AACCAACTAA TTGGTTGATT	ATCTAAGACT TAGATTCTGA
32451	TCCTGTCCCG	GGAGAAAAT	ATTTGAGTCG	CCACAACTTG GGTGTTGAAC	CTATAATIGA
32501	TGTTGTTTCC	GGAAATGAAC	AAATGTCGAA	CAAACAATTC GTTTGTTAAG	GTTTTTCGAA
32551	CTCCAATTGG	ATTCGTGACG	GTTCCCCAAC	ATGTTTGACG TACAAACTGC	GATGTCGGTA
32601	TCGGTAATTA	CGTCCTCTAC	CCGAACTTAA	TGGTTCACCT ACCAAGTGGA	TTACGTGGTT.
32651	TGTGTTTAGG	GGAGTTTTGT	TTTTAACCGG	TACCGGATCT	ATTTGATTCA TAAACTAAGT
32701	TTGTTCCGAT	ACCAAGGATT	TGATCCTTGA		AACTGTCGTG
32751	TCCACGGTAA	TGTCATCCTT	TGTTTTTATT	ACTATTCGAT	ACTTTGTGGA TGAAACACCT
32801	GGTGTGGTCG	AGGTAGAGGA	TTGACATCT	; ATTTACGTCT	GAAAGATGCT CTTTCTACGA
32851	TTTGAGTGAA	ACCAGAATTG	TTTTACACCO	TCAGTTTATE	TTGCTACAGT AACGATGTCA
32901	AAGTCAAAAC	CGACAATTTC	CGTCAAACC	AGGTTATAGA	GGAACAGTTC CCTTGTCAAG
32951	TTTCACGAG	AGAATAATA	TCTAAACTG(TTTTACCTC	GCTACTAAAC CGATGATTTG
	TTAAGGAAG	ACCTGGGTC	TATAACCTT	S AAATCTTIA	GAGATCTTAC CTCTAGAATG
	ACTTCCGTG	r CGGATATGT	r TGCGACAAC	C TAAATACGG	A ACCTATCAG A TTGGATAGTC
	GAATAGGTT	TAGAGTGCC	A TTTTGACGG	T TTTCATTGT	TGTCAGTCAA A ACAGTCAGTT
33151	GTTTACTTA CAAATGAAT	A ACGGAGACA T TGCCTCTGT	A AACTAAACC T TTGATTTGG	T GTAACACTA A CATTGTGAT	A CCATTACACT T GGTAATGTGA

Figure 26 AI

33251	CATTTTCATG	GGACTGGTCT	GGCCACAACT	ACATTAATGA	AATATTTGCC
	GTAAAAGTAC	CCTGACCAGA	CCGGTGTTGA	TGTAATTACT	TTATAAACGG
33301				CAAGAATAAA GTTCTTATTT	
33351	TGTTATGTTT	CAACGTGTTT	ATTTTTCAAT	TGCAGAAAAT	TTCAAGTCAT
	ACAATACAAA	GTTGCACAAA	TAAAAAGTTA	ACGTCTTTTA	AAGTTCAGTA
33401				CATAGCTTAT GTATCGAATA	
33451				ATTCAACCTG TAAGTTGGAC	
33501	CCCAACACAC	AGAGTACACA	GTCCTTTCTC	CCCGGCTGGC	CTTAAAAAGC
	GGGTTGTGTG	TCTCATGTGT	CAGGAAAGAG	GGGCCGACCG	GAATTTTTCG
33551	ATCATATCAT	GGGTAACAGA	CATATTCTTA	GGTGTTATAT	TCCACACGGT
	TAGTATAGTA	CCCATTGTCT	GTATAAGAAT	CCACAATATA	AGGTGTGCCA
33601	TTCCTGTCGA	GCCAAACGCT	CATCAGTGAT	ATTAATAAAC	TCCCCGGGCA
	AAGGACAGCT	CGGTTTGCGA	GTAGTCACTA	TAATTATTTG	AGGGGCCCGT
33651	GCTCACTTAA	GTTCATGTCG	CTGTCCAGCT	GCTGAGCCAC	AGGCTGCTGT
	CGAGTGAATT	CAAGTACAGC	GACAGGTCGA	CGACTCGGTG	TCCGACGACA
33701	CCAACTTGCG	GTTGCTTAAC	GGGCGGCGAA	GGAGAAGTCC	ACGCCTACAT
	GGTTGAACGC	CAACGAATTG	CCCGCCGCTT	CCTCTTCAGG	TGCGGATGTA
33751	GGGGGTAGAG	TCATAATCGT	GCATCAGGAT	AGGGCGGTGG	TGCTGCAGCA
	CCCCCATCTC	AGTATTAGCA	CGTAGTCCTA	TCCCGCCACC	ACGACGTCGT
33801	GCGCGCGAAT	AAACTGCTGC	CGCCGCCGCT	CCGTCCTGCA	GGAATACAAC
	CGCGCGCTTA	TTTGACGACG	GCGGCGGCGA	GGCAGGACGT	CCTTATGTTG
33851	ATGGCAGTGG	TCTCCTCAGC	GATGATTCGC	ACCGCCCGCA	GCATAAGGCG
	TACCGTCACC	AGAGGAGTCG	CTACTAAGCG	TGGCGGGCGT	CGTATTCCGC
33901	CCTTGTCCTC	CGGGCACAGC	AGCGCACCCT	GATCTCACTT	AAATCAGCAC
	GGAACAGGAG	GCCCGTGTCG	TCGCGTGGGA	CTAGAGTGAA	TTTAGTCGTG
33951	AGTAACTGCA	GCACAGCACC	ACAATATTGT	TCAAAATCCC	ACAGTGCAAG
	TCATTGACGT	CGTGTCGTGG	TGTTATAACA	AGTTTTAGGG	TGTCACGTTC
34001	GCGCTGTATC	CAAAGCTCAT	GGCGGGGACC	ACAGAACCCA	CGTGGCCATC
	CGCGACATAG	GTTTCGAGTA	CCGCCCCTGG	TGTCTTGGGT	GCACCGGTAG
34051	ATACCACAAG	CGCAGGTAGA	TTAAGTGGCG	ACCCCTCATA	AACACGCTGG
	TATGGTGTTC	GCGTCCATCT	AATTCACCGC	TGGGGAGTAT	TTGTGCGACC
34101	ACATAAACAT	TACCTCTTTT	GGCATGTTGT	AATTCACCAC	CTCCCGGTAC
	TGTATTTGTA	ATGGAGAAAA	CCGTACAACA	TTAAGTGGTG	GAGGGCCATG



34201	CCTCCCCAAA	ACCTGCCCGC	CGGCTATACA	CTGCAGGGAA	CCGGGACTGG
34201	CCACCCSTTT	TGGACGGGCG	GCCGATATGT	GACGTCCCTT	GGCCCTGACC
34251	AACAATGACA	GTGGAGAGCC	CAGGACTCGT	AACCATGGAT	CATCATGCTC
74274	TTGTTACTGT	CACCTCTCGG	GTCCTGAGCA	TTGGTACCTA	GTAGTACGAG
34301	GTCATGATAT	CAATGTTGGC	ACAACACAGG	CACACGTGCA	TACACTTCCT
54501	CAGTACTATA	GTTACAACCG	TGTTGTGTCC	GTGTGCACGT	atgtgaagga
34351	CAGGATTACA	AGCTCCTCCC	GCGTTAGAAC	CATATCCCAG	GGAACAACCC
	GTCCTAATGT	TCGAGGAGGG	CGCAATCTTG	GTATAGGGTC	CCTTGTTGGG
				>000>>C>C	TO COLACCE A A
34401	ATTCCTGAAT	CAGCGTAAAT	CCCACACTGC	AGGGAAGACC	ACCCACGIAG
	TAAGGACTTA	GTCGCATTTA	GGGTGTGACG	Tecentries	AGCG1GCA11
		CC A TODOTO A A	AGTGTTACAT	TCGGGCAGCA	GCGGATGATC
34451	CTCACGTTGT	CCMIIGICAA	TCACAATGTA	AGCCCGTCGT	CGCCTACTAG
	GAGTGCAACA	CGIAACAGII	1CAC/BITOTI		
34501	CTCCACTATC	GTAGCGCGGG	TTTCTGTCTC	AAAAGGAGGT	AGACGATCCC
34501	CACCTCATAC	CATCGCGCCC	AAAGACAGAG	TTTTCCTCCA	TCTGCTAGGG
	•				
34551	TACTGTACGG	AGTGCGCCGA	GACAACCGAG	ATCGTGTTGG	TCGTAGTGTC
34331	ATGACATGCC	TCACGCGGCT	CTGTTGGCTC	TAGCACAACC	AGCATCACAG
				•	
34601	ATGCCAAATG	GAACGCCGGA	CGTAGTCATA	TTTCCTGAAG	CAAAACCAGG
••••	. TACGGTTTAC	CTTGCGGCCT	GCATCAGTAT	AAAGGACTTC	GTTTTGGTCC
34651	TGCGGGCGTG	ACAAACAGAT	CTGCGTCTCC	GGTCTCGCCG	CTTAGATCGC
	ACGCCCGCAC	TGTTTGTCTA	GACGCAGAGG	CCAGAGCGGC	GAATCTAGUG
				000111001	CCAGGGGGGCC
34701	TCTGTGTAGT	AGTTGTAGTA	TATCCACTCT	CICAAAGCAI	CCTCCCCCC
	AGACACATCA	TCAACATCAT	ATAGGTGAGA	GAGIIICGIA	GG1CCGCGG
		a common ma mon	እ እ እ ርጥር ርጥጥር	ATGCGCCGCT	GCCCTGATAA
34751	CCTGGCTTCG	GGTTCTATGT	TTTGAGGAAG	TACCCCCCGA	CGGGACTATT
	GGACCGAAGC	CCAAGATACA	1110000000	17.000000	
24001	C > TC C > C C > C	CCCAGAATAA	GCCACACCCA	GCCAACCTAC	ACATTCGTTC
34801	CMICCACCAC	CCCTCTTATT	CGGTGTGGGT	CGGTTGGATG	TGTAAGCAAG
34851	TECGAGTCAC	ACACGGGAGG	AGCGGGAAGA	GCTGGAAGAA	CCATGTTTTT
34031	ACGCTCAGTG	TGTGCCCTCC	TOGCCCTTCT	CGACCTTCTI	GGTACAAAAA
34901	TTTTTTATTC	CAAAAGATTA	TCCAAAACCT	CAAAATGAAG	ATCTATTAAG
	AAAAAATAAG	GTTTTCTAAT	· AGGTTTTGGA	GTTTTACTTC	TAGATAATTC
34951	TGAACGCGCT	CCCCTCCGGI	GGCGTGGTCA	AACTCTACAC	CCAAAGAACA
	ACTTGCGCGA	GGGGAGGCCA	CCGCACCAGI	TIGAGATGI	GGTTTCTTGT
				, CCCUMCC * * 1	א ארכרא אארכים
35001	GATAATGGCA	TTTGTAAGAT	GITGCACAA!	CCCVVCCUM	A AGGCAAACGG
	CTATTACCGT	AAACATICTA	LAACGIGITA	CCGWGG11	TCCGTTTGCC
			- ምክአአርርርጥኣ፣	אררריזידר אַנּיִּי	GTGAATCTCC
35051	CCCTCACGT	CAAGTGGACC	· NAMAGGCIAN	, ACCC11CAG	CACTTAGAGG
	GGGAGTGCA	, GTTCACCTG	, ALLICCOALI	, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	

Figure 26 AK

35151	CCACCTTCTC	AATATATCTC	TAAGCAAATC	CCGAATATTA	AGTCCGGCCA
	GGTGGAAGAG	TTATATAGAG	ATTCGTTTAG	GGCTTATAAT	TCAGGCCGGT
35201				CCTTCAGCCT GGAAGTCGGA	
35251	ATCATGATTG	CAAAAATTCA	GGTTCCTCAC	AGACCTGTAT	AAGATTCAAA
	TAGTACTAAC	GTTTTTAAGT	CCAAGGAGTG	TCTGGACATA	TTCTAAGTTT
35301	AGCGGAACAT	TAACAAAAAT	ACCGCGATCC	CGTAGGTCCC	TTCGCAGGGC
	TCGCCTTGTA	ATTGTTTTTA	TGGCGCTAGG	GCATCCAGGG	AAGCGTCCCG
35351	CAGCTGAACA	TAATCGTGCA	GGTCTGCACG	GACCAGCGCG	GCCACTTCCC
	GTCGACTTGT	ATTAGCACGT	CCAGACGTGC	CTGGTCGCGC	CGGTGAAGGG
35401	CGCCAGGAAC	CATGACAAAA	GAACCCACAC	TGATTATGAC	ACGCATACTC
	GCGGTCCTTG	GTACTGTTTT	CTTGGGTGTG	ACTAATACTG	TGCGTATGAG
35451	CCTCGATACG	ATTGGTCGCA	TCGGGGCTAC	TAAGCTTGTT ATTCGAACAA	CGTACCCGCC
35501	CGATATAAAA	TGCAAGGTGC	TGCTCAAAAA	ATCAGGCAAA	GCCTCGCGCA
	GCTATATTTT	ACGTTCCACG	ACGAGTTTTT	TAGTCCGTTT	CGGAGCGCGT
35551	AAAAAGAAAG	CACATCGTAG	TCATGCTCAT	GCAGATAAAG	GCAGGTAAGC
	TTTTTCTTTC	GTGTAGCATC	AGTACGAGTA	CGTCTATTTC	CGTCCATTCG
35601				TTTCTCTCAA AAAGAGAGTT	
35651				CAAAAAAACA GTTTTTTTGT	
35701	AGAAGCCTGT	CTTACAACAG	GAAAAACAAC	CCTTATAAGC	ATAAGACGGA
	TCTTCGGACA	GAATGTTGTC	CTTTTTGTTG	GGAATATTCG	TATTCTGCCT
35751	CTACGGCCAT	GCCGGCGTGA	CCGTAAAAAA	ACTGGTCACC	GTGATTAAAA
	GATGCCGGTA	CGGCCGCACT	GGCATTTTTT	TGACCAGTGG	CACTAATTTT
35801				GGAGTCATAA CCTCAGTATT	
35851	GGTAAACACA	TCAGGTTGAT	TCACATCGGT	CAGTGCTAAA	AAGCGACCGA
	CCATTTGTGT	AGTCCAACTA	AGTGTAGCCA	GTCACGATTT	TTCGCTGGCT
35901	AATAGCCCGG	GGGAATACAT	ACCCGCAGGC	GTAGAGACAA	CATTACAGCC
	TTATCGGGCC	CCCTTATGTA	TGGGCGTCCG	CATCTCTGTT	GTAATGTCGG
35951	CCCATAGGAG	GTATAACAAA	ATTAATAGGA	GAGAAAAACA	CATAAACACC
	GGGTATCCTC	CATATTGTTT	TAATTATCCT	CTCTTTTTGT	GTATTTGTGG
36001	TGAAAAACCC	TCCTGCCTAG	GCAAAATAGC	ACCCTCCCGC	TCCAGAACAA
	ACTTTTTGGG	AGGACGGATC	CGTTTTATCG	TGGGAGGGCG	AGGTCTTGTT

rigure 26 AL

36101	AAAGAAAACC TTTCTTTTGG	ТАТТААААА ТТТТТТТААТА	ACACCACTCG TGTGGTGAGC	ACACGGCACC TGTGCCGTGG	AGCTCAATCA TCGAGTTAGT
36151		TTTTTTCCCG	GTTCACGTCT	CGCTCATATA	TATCCTGATT
36201	AAAATGACGT TTTTACTGCA	AACGGTTAAA TTGCCAATTT	GTCCACAAAA CAGGTGTTTT	AACACCCAGA TTGTGGGTCT	AAACCGCACG TTTGGCGTGC
36251		GGGTCTTTGC	TTTCGGTTTT	TTGGGTGTTG	AAGGAGTTTA
36301	CGTCACTTCC GCAGTGAAGG	GTTTTCCCAC CAAAAGGGTG	GTTACGTCAC CAATGCAGTG	TTCCCATTTT AAGGGTAAAA	AAGAAAACTA TTCTTTTGAT
36351	CAATTCCCAA GTTAAGGGTT	CACATACAAG GTGTATGTTC	TTACTCCGCC AATGAGGCGG	CTAAAACCTA GATTTTGGAT	CGTCACCCGC GCAGTGGGCG
36401	CCCGTTCCCA GGGCAAGGGT	CCCCCCCCCC	CACGTCACAA GTGCAGTGTT	ACTCCACCCC TGAGGTGGGG	CTCATTATCA GAGTAATAGT
					PacI
36451	TATTGGCTTC ATAACCGAAG	AATCCAAAAT TTAGGTTTTA	AAGGTATATT TTCCATATAA	ATTGATGATG TAACTACTAC	TTAATTAAGA AATTAATTCT
36501	ATTCGGATCT TAAGCCTAGA	GCGACGCGAG CGCTGCGCTC	GCTGGATGGC CGACCTACCG	CTTCCCCATT GAAGGGGTAA	ATGATTCTTC TACTAAGAAG
36551	TCGCTTCCGG AGCGAAGGCC	CGGCATCGGG GCCGTAGCCC	ATGCCCGCGT TACGGGCGCA	TGCAGGCCAT ACGTCCGGTA	GCTGTCCAGG CGACAGGTCC
36601	CAGGTAGATG GTCCATCTAC	ACGACCATCA TGCTGGTAGT	GGGACAGCTT CCCTGTCGAA	CAAGGCCAGC GTTCCGGTCG	AAAAGGCCAG TTTTCCGGTC
36651	GAACCGTAAA CTTGGCATTT	AAGGCCGCGT TTCCGGCGCA	TGCTGGCGTT	TTTCCATAGO AAAGGTATCO	CTCCGCCCCC CAGGCGGGGG
36701	CTGACGAGCA GACTGCTCGT	TCACAAAAA? AGTGTTTTT?	CGACGCTCAP CCTGCGAGTT	CAGTCTCCAC	GCGAAACCCG CGCTTTGGGC
36751	ACAGGACTAT TGTCCTGATA	AAAGATACCA TTTCTATGG	GGCGTTTCCC	CCTGGAAGCT GGACCTTCG	CCCTCGTGCG A GGGAGCACGC
36801	CTCTCCTGTT GAGAGGACAA	CCGACCCTG(CGCTTACCGC	ATACCTGTCC TATGGACAG	GCCTTTCTCC GCGAAAGAGG
36851	CTTCGGGAAC GAAGCCCTTC	GCACCGCGAL	T TCTCATAGCT A AGAGTATCG	r Cacgetgta A gtgegaeat	G GTATCTCAGT C CATAGAGTCA
36901	TCGGTGTAG(TCGTTCGCT	CAAGCTGGGG GGTTCGACCC	C TGTGTGCACGGGGGACGTGG	G AACCCCCCGT C TTGGGGGGCA

Figure 26 AM

37001	CGGTAAGACA	CGACTTATCG	CCACTGGCAG	CAGCCACTGG	TAACAGGATT
	GCCATTCTGT	GCTGAATAGC	GGTGACCGTC	GTCGGTGACC	ATTGTCCTAA
37051				GAGTTCTTGA	
	TCGTCTCGCT	CCATACATCC	GCCACGATGT	CTCAAGAACT	TCACCACCGG
37101	TAACTACGGC	TACACTAGAA	GGACAGTATT	TGGTATCTGC	GCTCTGCTGA
	ATTGATGCCG	ATGTGATCTT	CCTGTCATAA	ACCATAGACG	CGAGACGACT
37151				GCTCTTGATC	
				CGAGAACTAG	
37201	ACCACCGCTG	GTAGCGGTGG	TTTTTTTGTT	TGCAAGCAGC	AGATTACGCG
	TGGTGGCGAC	CATCGCCACC	AAAAAAACAA	ACGTTCGTCG	TCTAATGCGC
37251				GATCTTTTCT	
				CTAGAAAAGA	
37301				GGATTTTGGT	
•				CCTAAAACCA	
37351	TCAAAAAGGA	TCTTCACCTA	GATCCTTTTA	AATCAATCTA	AAGTATATAT
				TTAGTTAGAT	
37401				TTAATCAGTG	
				AATTAGTCAC	
37451	CTCAGCGATC	TGTCTATTTC	GTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG
				TCAACGGACT	
37501				CATCTGGCCC	
				GTAGACCGGG	
37551	ATGATACCGC.	GAGACCCACG	CTCACCGGCT	CCAGATTTAT	CAGCAATAAA
				GGTCTAAATA	
37601				TGGTCCTGCA	
				ACCAGGACGT	
37651	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG
				TTCGATCTCA	
37701	CCAGTTAATA	GTTTGCGCAA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT
	-			TAACGATGTC	
37751	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT
				GTCGAGGCCA	
37801	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC
				CGTTTTTTCG	
37851	TTCGGTCCTC	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT
	AAGCCAGGAG	GCTAGCAACA	GTCTTCATTC	AACCGGCGTC	ACAATAGTGA

Figure 26 AN

37951	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG
31931	CTACGAAAAG	ACACTGACCA	CTCATGAGTT	GGTTCAGTAA	GACTCTTATC
38001	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAACAC	GGGATAATAC
50001	ACATACGCCG	CTGGCTCAAC	GAGAACGGGC	CGCAGTTGTG	CCCTATTATG
38051	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTGGA	AAACGTTCTT
	GCGCGGTGTA	TCGTCTTGAA	ATTTTCACGA	GTAGTAACCT	TTTGCAAGAA
38101	CGGGGCGAAA	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG
	GCCCCGCTTT	TGAGAGTTCC	TAGAATGGCG	ACAACTCTAG	GTCAAGCTAC
38151	TAACCCACTC	GTGCACCCAA	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG
	ATTGGGTGAG	CACGTGGGTT	GACTAGAAGT	CGTAGAAAAT	GAAAGIGGIC
			a. ac acc	*********	AAAAAGGGAA
38201	CGTTTCTGGG	TGAGCAAAAA	CAGGAAGGCA	TTTACGGCGT	TTTTTTTCCCTT
	GCAAAGACCC	ACTCGTTTTT	GTCCTTCCGT	TITACGGCGT	1111100011
	TAAGGGCGAC	10001110T	ጥሮ አ አ ጥ አ ሮ ጥሮ አ	ተ ልር ተር ተርርር	TTTTCAATAT
38251	TAAGGGCGAC	MCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	ACTTATEACT	ATGAGAAGGA	AAAAGTTATA
	ATTCCCGCTG	IGCCITIACA	ACTIATOROL	71.0.10.10.0	
20201	TATTGAAGCA	ተተሞል ጥር ል GGG	TTATTGTCTC	ATGAGCGGAT	ACATATTTGA
38301	TATTGARGER	AAATAGTCCC	AATAACAGAG	TACTCGCCTA	TGTATAAACT
38351	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA
30331	TACATAAATC	TTTTTATTTG	TTTATCCCCA	AGGCGCGTGT	AAAGGGGCTT
38401	AAGTGCCACC	TGACGTCTAA	GAAACCATTA	TTATCATGAC	ATTAACCTAT
• • • • • •	TTCACGGTGG	ACTGCAGATT	CTTTGGTAAT	AATAGTACTG	TAATTGGATA
38451	AAAAATAGGC	GTATCACGAG	GCCCTTTCGT	CTTCAAGAAT	TGGATCCGAA
	TTTTTATCCG	CATAGTGCTC	CGGGAAAGCA	GAAGTTCTTA	ACCTAGGCTT
		PacI			
			(CDO ID NO	221	
38501	TTCTTAATTT	CTTAATTAA	(SEQ ID NO): <i>34]</i> \.33\	
	AAGAATTAAA	GAATTAATT	(SEQ ID NO	1.331	

Figure 26 AO

1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	ACTATTACTC
51	GGGGTGGAGT	TTGTGACGTG	GCGCGGGGCG	TGGGAACGGG	GCGGGTGACG
				ACCCTTGCCC	
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
				CACACCGCCT	
151	GCGACGGATG	TGGCAAAAGT	GACGTTTTTG	GTGTGCGCCG	GTGTACACAG
				CACACGCGGC	
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
				CTACAACATC	
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
				CCCTTTTGAC	
301	AGTGAAATCT	GAATAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
				ATCGCGCATT	
351	GGGCCGCGG	GACTTTGACC	GTTTACGTGG	AGACTCGCCC	AGGTGTTTTT
				TCTGAGCGGG	
401	CTCAGGTGTT	TTCCGCGTTC	CGGGTCAAAG	TTGGCGTTTT	ATTATTATAG
				AACCGCAAAA	
451	GCGGCCGCGA	TCCATTGCAT	ACGTTGTATC	CATATCATAA	TATGTACATT
				GTATAGTATT	
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
	•			ACAACTGTAA	
551	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
		•		TAATCAAGTA	
601	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG
				TACCGGGCGG	
651	CCCAACGACC	CCCGCCCATT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
				TACTGCATAC	
701	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAG	TATTTACGGT
				TACCCACCTC	
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
				TAGTATACGG	
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	GGATAACTGC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT

Figure 27A

					maaaaamaa
901	TCGCTATTAC	CATGGTGATG	CGGTTTTGGC	AGTACATCAA	TGGGCGTGGA
	AGCGATAATG	GTACCACTAC	GCCAAAACCG	TCATGTAGTT	ACCCGCACCT
951	TACCGGTTTG	ACTCACGGGG	ATTTCCAAGT	CTCCACCCCA	TTGACGTCAA
221	MCCCCAAAC	TCACTCCCC	TAAAGGTTCA	GAGGTGGGGT	AACTGCAGTT
	AICGCCAAAC	198919000	.,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	•	
				CC3 CMMTCC3	A A A TICTIC CTA
1001	TGGGAGTTTG	TTTTGGCACC	AAAATCAACG	GGACTTICCA	MANIGICGIA
	ACCCTCAAAC	AAAACCGTGG	TTTTAGTTGC	CCTGAAAGGT	TTTACAGCAT
1051	ACAACTCCGC	CCCATTGACG	CAAATGGGCG	GTAGGCGTGT	ACGGTGGGAG
•	TGTTGAGGCG	GGGTAACTGC	GTTTACCCGC	CATCCGCACA	TGCCACCCTC
	.0.,0				
1101	ርጥርጥ አጥ አጥ አ	GCAGAGCTCG	TTTAGTGAAC	CGTCAGATCG	CCTGGAGACG
1101	GICIAINIAN	OCHOROCICO	AAATCACTTG	CCACTCTACC	GGACCTCTGC
	CAGATATATT	CGICICGAGC	AAAICACIIG	001101011100	•••••
				>0>000000	CCATCCACCC
1151	CCATCCACGC	TGTTTTGACC	TCCATAGAAG	ACACCGGGAC	CONTCCAGCC
	GGTAGGTGCG	ACAAAACTGG	AGGTATCTTC	TGTGGCCCTG	GCTAGGTCGG
1201	TCCGCGGCCG	GGAACGGTGC	ATTGGAACGC	GGATTCCCCG	TGCCAAGAGT .
	AGGCGCCGGC	CCTTGCCACG	TAACCTTGCG	CCTAAGGGGC	ACGGTTCTCA
	AGGCGCCGG	••••			
1051		ACCATIGCECG	GCAAGTGGTC	CAAGAGGTCC	GTGCCCGGCT
1251	GAGA:CIGCC	MCCM1GGCCG	CGTTCACCAG	GTTCTCCAGG	CACGGGCCGA
	CTCTAGACGG	1661ACCGGC	CGIICACCAG	011010000	0.7000000
			. = 0. 00. 000	0003000000	CCCCCACAGG
1301	GGTCCACCGT	GAGGGAGAGG	ATGAGGAGGG	CCGAGCCCGC	COCCORCACO
	CCAGGTGGCA	CTCCCTCTCC	TACTCCTCCC	GGCTCGGGCG	6666616166
1351	GTGAGGAGGA	CCGAGCCCGC	CGCAGTGGGC	GTGGGCGCCG	TGTCCAGGGA
	CACTCCTCCT	GGCTCGGGCG	GCGTCACCCG	CACCCGCGGC	ACAGGTCCCT
	C.1.C.1.C.1.C.1				
1 401	CCTCCACAAC	CACGGGGGCA	TCACCTCCTC	CAACACCGCC	GCCACCAACG
1401	CCIGGAGAAG	CHCCCCCCC	AGTGGAGGAG	GTTGTGGGGG	CGGTGGTTGC
	GGACCTCTTC	GIGCCGCGGI	AG 1 GGAGGAG	01.01.000	••••
			0000100100	ACCACCACCT	GGGCTTCCCC
1451	CCGACTGCGC	CTGGCTGGAG	GCCCAGGAGG	MCGMGGMGG1	00000770000
	GGCTGACGCG	GACCGACCTC	CGGGTCCTCC	TGCTCCTCCA	CCCGAAGGGG
1501	GTGAGGCCCC	AGGTGCCCCT	GAGGCCCATG	ACCTACAAGG	GCGCCGTGGA
	CACTCCGGGG	TCCACGGGGA	CTCCGGGTAC	TGGATGTTCC	CGCGGCACCT
	0				
1551	CCTCTCCCAC	TTCCTGAAGG	AGAAGGGCGG	CCTGGAGGGC	CTGATCCACT
1221	CCIGICCCAC	A A COA CETTOO	一型で型型ででして	GGACCTCCCG	GACTAGGTGA
	GGACAGGGTG	MAGGACTICC	101100000	00	
					CACCCAGGGC
1601	CCCAGAAGAG	GCAGGACATC	CIGGACCIGI	GGGIGIACCA	CACCCAGGGC
	GGGTCTTCTC	CGTCCTGTAG	GACCTGGACA	CCCACATGGT	GTGGGTCCCG
1651	TACTTCCCCG	ACTGGCAGAA	CTACACCCCC	GGCCCCGGCA	TCAGGTTCCC
	ATCAAGGGG	TGACCGTCTI	GATGTGGGGG	CCGGGGCCGT	AGTCCAAGGG
	*** 0.5400000				
		CCCTCCTCCT	י ייירא אכריייכניי	GCCCGTGGAG	CCCGAGAAGG
1/01	CCIGACCITC	0001001001	ACTITICATO	CGGGCACCTC	GGGCTCTTCC
	GGACTGGAAG	CCGACCACGA	AUTICONCE	COOGCACCIC	
		= =			
1751	TGGAGGAGGC	CAACGAGGGC	GAGAACAACI	GCGCCGCCCA	CCCCATGTCC
	ACCTCCTCCG	GTTGCTCCC	CTCTTGTTG?	CGCGGCGGG1	GGGGTACAGG

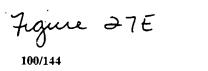
Figure 27B

1851				GGAGCTGCAC CCTCGACGTG	
1901				CTGTGCCTTC GACACGGAAG	
1951				TCCTTGACCC AGGAACTGGG	
2001				GGAAATTGCA CCTTTAACGT	
2051				GGGTGGGGCA CCCACCCCGT	
2101	GGGGAGGATT	GGGAAGACAA	TAGCAGGCAT	GCTGGGGATG	CGGTGGGCTC
	CCCCTCCTAA	CCCTTCTGTT	ATCGTCCGTA	CGACCCCTAC	GCCACCCGAG
2151	TATGGCCGAT ATACCGGCTA	CGCGCGCGC	TACTGAAATG ATGACTTTAC	TGTGGGCGTG ACACCCGCAC	GCTTAAGGGT CGAATTCCCA
2201	GGGAAAGAAT	ATATAAGGTG	GGGGTCTTAT	GTAGTTTTGT	ATCTGTTTTG
	CCCTTTCTTA	TATATTCCAC	CCCCAGAATA	CATCAAAACA	TAGACAAAAC
2251	CAGCAGCCGC	CGCCGCCATG	AGCACCAACT	CGTTTGATGG	AAGCATTGTG
	GTCGTCGGCG	GCGGCGGTAC	TCGTGGTTGA	GCAAACTACC	TTCGTAACAC
2301	AGCTCATATT	TGACAACGCG	CATGCCCCCA	TGGGCCGGGG	TGCGTCAGAA
	TCGAGTATAA	ACTGTTGCGC	GTACGGGGGT	ACCCGGCCCC	ACGCAGTCTT
2351	TGTGATGGGC	TCCAGCATTG	ATGGTCGCCC	CGTCCTGCCC	GCAAACTCTA
	ACACTACCCG	AGGTCGTAAC	TACCAGCGGG	GCAGGACGGG	CGTTTGAGAT
2401				CGCCGTTGGA GCGGCAACCT	
2451	TCCGCCGCCG	CTTCAGCCGC	TGCAGCCACC	GCCCGCGGGA	TTGTGACTGA
	AGGCGGCGGC	GAAGTCGGCG	ACGTCGGTGG	CGGGCGCCCT	AACACTGACT
2501	CTTTGCTTTC	CTGAGCCCGC	TTGCAAACAĞ	TGCAGCTTCC	CGTTCATCCG
	GAAACGAAAG	GACTCGGGCG	AACGTTTGTC	ACGTCGAAGG	GCAAGTAGGC
2551	CCCGCGATGA	CAAGTTGACG	GCTCTTTTGG	CACAATTGGA	TTCTTTGACC
	GGGCGCTACT	GTTCAACTGC	CGAGAAAACC	GTGTTAACCT	AAGAAACTGG
2601	CGGGAACTTA	ATGTCGTTTC	TCAGCAGCTG	TTGGATCTGC	GCCAGCAGGT
	GCCCTTGAAT	TACAGCAAAG	AGTCGTCGAC	AACCTAGACG	CGGTCGTCCA
2651	TTCTGCCCTG	AAGGCTTCCT	CCCCTCCCAA	TGCGGTTTAA	AACATAAATA
	AAGACGGGAC	TTCCGAAGGA	GGGGAGGGTT	ACGCCAAATT	TTGTATTTAT
2701	AAAAACCAGA	CTCTGTTTGG	ATTTGGATCA	AGCAAGTGTC	TTGCTGTCTT
	TTTTTGGTCT	GAGACAAACC	TAAACCTAGT	TCGTTCACAG	AACGACAGAA

Figure 27C

2751	TATTTAGGGG T	TTTGCGCGC (GCGGTAGGCC	CGGGACCAGC	GGTCTCGGTC CCAGAGCCAG
	ATAAATCCCC A				
2801	GTTGAGGGTC C'	TGTGTATTT	TTTCCAGGAC	GTGGTAAAGG	TGACTCTGGA
	CAACTCCCAG G	ACACATAAA .	AAAGGTCCTG	CACCATTTCC	ACTGAGACCI
2851	TGTTCAGATA C	ATGGGCATA	AGCCCGTCTC	TGGGGTGGAG	GTAGCACCAC
	ACAAGTCTAT G	TACCCGTAT	TCGGGCAGAG	ACCCCACCTC	CATCGTGGTG
2901	TGCAGAGCTT C.	ATGCTGCGG	GGTGGTGTTG	TAGATGATCC	AGTCGTAGCA
2301	ACGTCTCGAA G	TACGACGCC	CCACCACAAC	ATCTACTAGG	TCAGCATCGT
2951	GGAGCGCTGG G	CGTGGTGCC	TAAAAATGTC	TTTCAGTAGC	AAGCTGATTG
2331	CCTCGCGACC C	GCACCACGG	ATTTTTACAG	AAAGTCATCG	TTCGACTAAC
3001	CCAGGGGCAG G	CCCTTGGTG	TAAGTGTTTA	CAAAGCGGTT	AAGCTGGGAT
3001	GGTCCCCGTC C	GGGAACCAC	ATTCACAAAT	GTTTCGCCAA	TTCGACCCTA
3051	GGGTGCATAC G	TGGGGATAT	GAGATGCATC	TTGGACTGTA	TTTTTAGGTT
3031	CCCACGTATG C	ACCCCTATA	CTCTACGTAG	AACCTGACAT	AAAAATCCAA
3101	GGCTATGTTC C	CAGCCATAT	CCCTCCGGGG	ATTCATGTTG	TGCAGAACCA
3202	CCGATACAAG G	GTCGGTATA	GGGAGGCCCC	TAAGTACAAC	ACGICITIGGI
3151	CCAGCACAGT G	TATCCGGTG	CACTTGGGAA	ATTTGTCATG	TAGCTTAGAA
5252	GGTCGTGTCA C	ATAGGCCAC	GTGAACCCTT	TAAACAGTAC	ATCGAATCTT
3201	GGAAATGCGT G	GAAGAACTT	GGAGACGCCC	TTGTGACCTC	CAAGATTTTC
3201	CCTTTACGCA C	CTTCTTGAA	CCTCTGCGGG	AACACTGGAG	GIICIAAAAG
3251	CATGCATTCG T	CCATAATGA	TGGCAATGGG	CCCACGGGCG	GCGGCCTGGG
3202	GTACGTAAGC A	AGGTATTACT	ACCGTTACCC	GGGTGCCCGC	CGCCGGACCC
3301	CGAAGATATT 1	TCTGGGATCA	CTAACGTCAT	AGTTGTGTTC	CAGGATGAGA
	GCTTCTATAA A	AGACCCTAGT	GATTGCAGTA	TCAACACAA	, GICCIACICI
3351	TCGTCATAGG (CCATTTTTAC	AAAGCGCGG	CGGAGGGTG	CAGACTGCGG
	AGCAGTATCC	GGTAAAAATG	TTTCGCGCCC	GCCTCCCACC	GICTGACGCC
2401	ጥእጥጾ እጥርር ሞጥ	CCATCCGGCC	CAGGGGCGT	A GTTACCCTC	A CAGATTTGCA
3401	ATATTACCAA	GGTAGGCCGG	GTCCCCGCA	r caatgggag'	r grctaaacgt
3451	TTTCCCACGC	TTTGAGTTCA	GATGGGGGG	A TCATGTCTA	CTGCGGGGG
3431	AAAGGGTGCG	AAACTCAAGT	CTACCCCCC	T AGTACAGAT	G GACGCCCCGC
3501	ATGAAGAAAA	CGGTTTCCGG	GGTAGGGGA	G ATCAGCTGG	G AAGAAAGCAG
	TACTTCTTTT	GCCAAAGGCC	CCATCCCCT	C TAGTEGACE	C 11C111CG1C
3551	GTTCCTGAGC	AGCTGCGACT	TACCGCAGC	C GGTGGGCCC	G TAAATCACAC
	CAAGGACTCG	TCGACGCTG	ATGGCGTCG	G CCACCCGGG	CAITIAGIGIG
3601	CTATTACCGG	CTGCAACTG	TAGTTAAGA	G AGCTGCAGC	T GCCGTCATCC
	GATAATGGCC	GACGTTGAC	2 ATCAATICT	C TCGACGICG	A COGCAGIAGO
3651	CTGAGCAGGG	GGGCCACTT	GTTAAGCAT	G TCCCTGACT	C GCATGTTTTC
5051	GACTCGTCCC	CCCGGTGAA	G CAATTCGTA	C AGGGACTGA	G CGTACAAAAG

3701					AGCAGTTCTT TCGTCAAGAA
3751					CGTAGGCATG GCATCCGTAC
3801					GCTCGGTCAC CGAGCCAGTG
3851			CCAGCATATC GGTCGTATAG		GCGGGTTGGG CGCCCAACCC
3901			GTAGTCGGTG CATCAGCCAC		CGGGCCAGGG GCCCGGTCCC
3951			AGGGTCCTCG TCCCAGGAGC		
4001			CTGCGCGCTG GACGCGCGAC		
4051			GCTGCCGGTC CGACGGCCAG		
4101			TCATAGTCCA AGTATCAGGT		
4151			GGAGGAGGCG CCTCCTCCGC		
4201			TGGGCGCGAG ACCCGCGCTC		
4251			CCGCAGACGG GGCGTCTGCC		CACGAGCCAG GTGCTCGGTC
4301			GTCAAAAACC CAGTTTTTGG		
4351			TTTCCATGAG AAAGGTACTC		
		CAGGCACAGG	GGCATATGTC	TGAACTCTCC	GGACAGGAGC
4451	AGCGGTGTTC TCGCCACAAG		CTCGTATAGA GAGCATATCT		
4501	AAAGGCTCGC TTTCCGAGCG		GCACGAAGGA CGTGCTTCCT		
4551	GGTCGTTGTC CCAGCAACAG		TCCACTCGCT AGGTGAGCGA		
4601	TCGCCCTCTT AGCGGGAGAA		GAAGGTGATŢ CTTCCACTAA		



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4701	CGTCCTCACT	CTCTTCCGCA	TCGCTGTCTG	CGAGGCCAG	CTGTTGGGGT
				GCTCCCGGTC	
4751	GAGTACTCCC	TCTGAAAAGC	GGGCATGACT	TCTGCGCTAA	GATTGTCAGT
	CTCATGAGGG	AGACTTTTCG	CCCGTACTGA	AGACGCGATT	CTAACAGTCA
4801	TTCCAAAAAC	GAGGAGGATT	TGATATTCAC	CTGGCCCGCG	GTGATGCCTT
•••	AAGGTTTTTG	CTCCTCCTAA	ACTATAAGTG	GACCGGGCGC	CACTACGGAA
4851	TGAGGGTGGC	CGCATCCATC	TGGTCAGAAA	AGACAATCTT	TTTGTTGTCA
	ACTCCCACCG	GCGTAGGTAG	ACCAGTCTTT	TCTGTTAGAA	AAACAACAGT
4901	AGCTTGGTGG	CAAACGACCC	GTAGAGGGCG	TTGGACAGCA	ACTTGGCGAT
	TCGAACCACC	GTTTGCTGGG	CATCTCCCGC	AACCTGTCGT	TGAACCGCTA
4951	GGAGCGCAGG	GTTTGGTTTT	TGTCGCGATC	GCCGCCTCC	TTGGCCGCGA
				CCGCGCGAGG	
5001	TGTTTAGCTG	CACGTATTCG	CGCGCAACGC	ACCGCCATTC	GGGAAAGACG
				TGGCGGTAAG	
5051	GTGGTGCGCT	CGTCGGGCAC	CAGGTGCACG	CGCCAACCGC	GGTTGTGCAG
				GCGGTTGGCG	
5101	GGTGACAAGG	TCAACGCTGG	TGGCTACCTC	TCCGCGTAGG	CGCTCGTTGG
				AGGCGCATCC	
5151	TCCAGCAGAG	GCGGCCGCCC	TTGCGCGAGC	AGAATGGCGG	TAGGGGGTCT
					ATCCCCCAGA
5201	AGCTGCGTCT	CGTCCGGGGG	GTCTGCGTCC	ACGGTAAAGA	CCCCGGGCAG
	TCGACGCAGA	GCAGGCCCCC	CAGACGCAGG	TGCCATTTCT	GGGGCCCGTC
5251	CAGGCGCGCG	TCGAAGTAGT	CTATCTTGCA	TCCTTGCAAG	TCTAGCGCCT
					AGATCGCGGA
5301	GCTGCCATGC	GCGGGCGGCA	AGCGCGCGCT	CGTATGGG11	GAGTGGGGGA
					CCAAATGTC
5351	CCCCATGGCA	TGGGGTGGGT	GAGCGCGGAG	CCCIMCMICC	CGCAAATGTC
					GCGTTACAG
5401	GTAAACGTAG	AGGGGCTCTC	TGAGTATICC	AAGATATGTA	GGGTAGCATC
					CCCATCGTAG
5451	TTCCACCGCG	GATGCTGGCG	CGCACGTAAT	CGTATAGTTC	GTGCGAGGGA
					CACGCTCCCAA
5501	GCGAGGAGGT	CGGGACCGAG	GTTGCTACGO	- 0000000000	CTGCTCGGAA
					GACGAGCCTT
5551	GACTATCTGO	CTGAAGATG	CATGTGAGT	r GGATGATATO	GTTGGACGCT
	CTGATAGACO	GACTTCTAC	GTACACTCA	A CCTACTATAC	CAACCTGCGA

Figure 27F

5651	GAGGCGTAGG CTCCGCATCC	AGTCGCGCAG TCAGCGCGTC	CTTGTTGACC GAACAACTGG	AGCTCGGCGG TCGAGCCGCC	TGACCTGCAC ACTGGACGTG
5701	GTCTAGGGCG CAGATCCCGC	CAGTAGTCCA GTCATCAGGT	GGGTTTCCTT CCCAAAGGAA	GATGATGTCA CTACTACAGT	TACTTATCCT ATGAATAGGA
5751				GGACAAACTC CCTGTTTGAG	
5801				GCCTCCGAAC CGGAGGCTTG	
5851				GGCGCAGCAT CCGCGTCGTA	
5901				GGAGCGAGGT CCTCGCTCCA	
5951				TACTGGTATT ATGACCATAA	
6001	CAGCAGCGTA	GGCGGGACGA	GGGTCTCGTT	AAAGTCCGTG TTTCAGGCAC	GCGAAAAACC
6051	TTGCGCCTAA	ACCGTCCCGC	TTCCACTGTA	CGTTGAAGAG GCAACTTCTC	ATAGAAAGGG
6101	CGCGCTCCGT	ATTTCAACGC	ACACTACGCC	AAGGGTCCCG TTCCCAGGGC	CGTGGAGCCT
6151	TGCCAACAAT	TAATGGACCC	GCCGCTCGTG	GATCTCGTCA CTAGAGCAGT	TTCGGCAACT
6201	ACAACACCGG	GTGTTACATT	TCAAGGTTCT	AGCGCGGGAT TCGCGCCCTA	CGGGAACTAC
6251	CTTCCGTTAA	AAAATTCAAG	GAGCATCCAC	AGCTCTTCAG TCGAGAAGTC	CCCTCGACTC
6301	GGGCACGAGA	CTTTCCCGGG	TCAGACGTTC	ATGAGGGTTG TACTCCCAAC	CTTCGCTGCT
		GTCCAGTGCC	CGGTAATCGT	AAACGTCCAC	CAGCGCTTTC
		CCGCTGGATA	CCGGTAAAAA	AGACCCCACT	ACGTCATCTT
		AGAACAAGGG	TCGCCAGGGT	AGGTTCCAAG	CGCCGATCCA
6501	CTCGCGCGGC GAGCGCGCCG	AGTCACTAGA TCAGTGATCT	GGCTCATCTC CCGAGTAGAG	CGCCGAACTT GCGGCTTGAA	CATGACCAGC GTACTGGTCG

Figure 27G

6601	TACATCGTAG ATGTAGCATC	GTGACAAAGA CACTGTTTCT	GACGCTCGGT CTGCGAGCCA	GCGAGGATGC CGCTCCTACG	GAGCCGATCG CTCGGCTAGC
6651	GGAAGAACTG CCTTCTTGAC	GATCTCCCGC CTAGAGGGCG	CACCAATTGG GTGGTTAACC	AGGAGTGGCT TCCTCACCGA	ATTGATGTGG TAACTACACC
6701	TGAAAGTAGA ACTTTCATCT	AGTCCCTGCG TCAGGGACGC	ACGGGCCGAA TGCCCGGCTT	CACTCGTGCT GTGAGCACGA	GGCTTTTGTA CCGAAAACAT
6751	AAAACGTGCG TTTTGCACGC	GTCATGACCG	TCGCCACGTG	CCCGACATGT	AGGACGTGCT
6801 .	GGTTGACCTG CCAACTGGAC	TGCTGGCGCG	TGTTCCTTCG	TCTCACCCTT	AAACTCGGGG
6851	TCGCCTGGCG AGCGGACCGC	CCAAACCGAC	CACCAGAAGA	TGAAGCCGAC	GAACAGGAAC
6901	ACCGTCTGGC TGGCAGACCG	ACGAGCTCCC	CTCAATGCCA	CCTAGCCTIG	TGGTGCGGCG
6951	CGCTCGGGTT	TCAGGTCTAC	AGGCGCGCGC	CGCCAGCCTC	
7001	TGTAGCGCGT	CTACCCTCGA	CAGGTACCAG	ACCTCGAGGG	
7051	GTCAGGCGGG CAGTCCGCCC	AGCTCCTGCA TCGAGGACGT	GGTTTACCTC CCAAATGGAG	GCATAGACGG CGTATCTGCC	GTCAGGGCGC CAGTCCCGCG
7101	GGGCTAGATC CCCGATCTAG	CAGGTGATAC GTCCACTATG	CTAATTTCCA GATTAAAGGT	GGGGCTGGTT	GGTGGCGGCG CCACCGCCGC
7151	TCGATGGCTT AGCTACCGAA	GCAAGAGGCC CGTTCTCCGG	GCATCCCCGC CGTAGGGGCG	GGCGCGACTA CCGCGCTGAT	CGGTACCGCG
7201	CGGCGGGCGG	TGGGCCGCGG ACCCGGCGCC	GGGTGTCCTT CCCACAGGAA	GGATGATGCA CCTACTACGT	TCTAAAAGCG AGATTTTCGC
7251	GTGACGCGGG CACTGCGCCC	CGAGCCCCCG	GAGGTAGGGG	GGGCTCCGGA	CCCGCCGGA
7301	GAGGGGGCAG CTCCCCCGTC	GGGCACGTCG	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CGGGCAGGAG CCCGTCCTC	CTGGTGCTGC GACCACGACG
7351	GCGCGTAGGT CGCGCATCCA	TGCTGGCGAA ACGACCGCTT	CGCGACGACG CGCTGCTGC	CGGCGGTTGA GCCGCCAACT	TCTCCTGAAT AGAGGACTTA
7401	CTGGCGCCTC GACCGCGGAG	TGCGTGAAGA ACGCACTTCT	COACGGCCC COOCCCCCCCCCCCCCCCCCCCCCCCCCCC	GGTGAGCTTG CCACTCGAAC	AACCTGAAAG TTGGACTTTC
7451	AGAGTTCGAC TCTCAAGCTC	AGAATCAATT	TCGGTGTCGT A AGCCACAGCA	TGACGGCGGG A ACTGCCGCC	CTGGCGCAAA GACCGCGTTT

Figure 27H

7551	CTGCTCGATC	TCTTCCTCCT	GGAGATCTCC	GCGTCCGGCT	CGCTCCACGG
	GACGAGCTAG	AGAAGGAGGA	CCTCTAGAGG	CGCAGGCCGA	GCGAGGTGCC
7601	TGGCGGCGAG	GTCGTTGGAA	ATGCGGGCCA	TGAGCTGCGA	GAAGGCGTTG
	ACCGCCGCTC	CAGCAACCTT	TACGCCCGGT	ACTCGACGCT	CTTCCGCAAC
7651	AGGCCTCCCT	CGTTCCAGAC	GCGGCTGTAG	ACCACGCCCC	CTTCGGCATC
				TGGTGCGGGG	
7701	GCGGGCGCGC	ATGACCACCT	GCGCGAGATT	GAGCTCCACG	TGCCGGGCGA
٠				CTCGAGGTGC	
7751	AGACGGCGTA	GTTTCGCAGG	CGCTGAAAGA	GGTAGTTGAG	GGTGGTGGCG
				CCATCAACTC	
7801	GTGTGTTCTG	CCACGAAGAA	GTACATAACC	CAGCGTCGCA	ACGTGGATTC
				GTCGCAGCGT	
7851	GTTGATATCC	CCCAAGGCCT	CAAGGCGCTC	CATGGCCTCG	TAGAAGTCCA
				GTACCGGAGC	
7901	CGGCGAAGTT	GAAAAACTGG	GAGTTGCGCG	CCGACACGGT	TAACTCCTCC
				GGCTGTGCCA	
7951	TCCAGAAGAC	GGATGAGCTC	GGCGACAGTG	TCGCGCACCT	CGCGCTCAAA
		·		AGCGCGTGGA	
8001	GGCTACAGGG	GCCTCTTCTT	CTTCTTCAAT	CTCCTCTTCC	ATAAGGGCCT
				GAGGAGAAGG	
8051	CCCCTTCTTC	TTCTTCTGGC	GGCGGTGGGG	GAGGGGGGAC	ACGGCGGCGA
				CTCCCCCTG	
8101	CGACGGCGCA	CCGGGAGGCG	GTCGACAAAG	CGCTCGATCA	TCTCCCCGCG
				GCGAGCTAGT	
8151	GCGACGGCGC	ATGGTCTCGG	TGACGGCGCG	GCCGTTCTCG	CCCCCCCCC
				CGGCAAGAGC	
8201	GTTGGAAGAC	GCCGCCCGTC	ATGTCCCGGT	TATGGGTTGG	CCCCCCC
				ATACCCAACC	
8251	CCATGCGGCA	GGGATACGGC	GCTAACGATG	CATCTCAACA	MANCANCACA
					TAACAACACA
8301	AGGTACTCCG	CCGCCGAGGG	ACCTGAGCGA	GTCCGCATCG	ACCCCTA CCC
					TGGCCTAGCC
8351	AAAACCTCTC	GAGAAAGGCG	TCTAACCAGT	CACAGTCGCA	AGGTAGGCTG
•					TCCATCCGAC
8401	AGCACCGTGG	CGGGCGGCAG	CGGGCGGCGG	TCGGGGTTGT	TTCTGGCGGA
	TCGTGGCACC	GCCCGCCGTC	GCCCGCCGCC	AGCCCCAACA	AAGACCGCCT

Figure 27I

8501	TCGACAGAAG	CACCATGTCC	TTGGGTCCGG	CCTGCTGAAT	GCGCAGGCGG
0301	ACCTCTCTTC	GTGGTACAGG	AACCCAGGCC	GGACGACTTA	CGCGTCCGCC
	AGCIGICILE				
0553	MOCCOCO NACC	ררכאבבריייר	GTTTTGACAT	CGGCGCAGGT	CTTTGTAGTA
8551	TCGGCCATGC	CCCAGGCIIC	CAAAACTGTA	CCCCCCTCCA	GAAACATCAT
	AGCCGGTACG	GGGTCCGAMG	CAAAACIGIA	GCCGCGTCCA	0.22.0
	_				ጥ ር ጥጥ ጥጥር ጥር
8601	GTCTTGCATG	AGCCTTTCTA	CCGGCACTTC	TTCTTCTCCT	1001011010
	CAGAACGTAC	TCGGAAAGAT	GGCCGTGAAG	AAGAAGAGGA	AGGAGAACAG
8651	CTGCATCTCT	TGCATCTATC	GCTGCGGCGG	CGGCGGAGTT	TGGCCGTAGG
	GACGTAGAGA	ACGTAGATAG	CGACGCCGCC	GCCGCCTCAA	ACCGGCATCC
8701	TEGECECETE	TTCCTCCCAT	GCGTGTGACC	CCGAAGCCCC	TCATCGGCTG
0,01	*CCCCCCCG*C	AAGGAGGGTA	CGCACACTGG	GGCTTCGGGG	AGTAGCCGAC
	Account				
8751	N N C C N C C C C C T	ACCTCCCCGA	CAACGCGCTC	GGCTAATATG	GCCTGCTGCA
8/31	MUCCUCCCC	TCCACCCCCT	GTTGCGCGAG	CCGATTATAC	CGGACGACGT
	TICGICCCGA	1CCAGCCGC1	011000000	•••	
	000000000000000000000000000000000000000	CCTACACTCC	AAGTCATCCA	TGTCCACAAA	GCGGTGGTAT
8801	CCTGCGTGAG	GGIAGACIGG	TTCAGTAGGT	ACAGGTGTTT	CCCCACCATA
	GGACGCACTC	CCATCIGACC	11CAGIAGG1	ACAGGIGITI	
			AGTGCAGTTG	CCCATAACGG	ХССХСТТААС
8851	GCGCCCGTGT	TGATGGTGTA	AGIGCAGIIG	CCCTATACCC	TCCTCAATTC
	CGCGGGCACA	ACTACCACAT	TCACGTCAAC	CGGIMIIGCC	1001041110
					OCCCA CTA A C
8901	GGTCTGGTGA	CCCGGCTGCG	AGAGCTCGGT	GTACCTGAGA	CGCGAGTAAG
	CCAGACCACT	GGGCCGACGC	TCTCGAGCCA	CATGGACTCT	GCGCTCATTC
8951	CCCTCGAGTC	AAATACGTAG	TCGTTGCAAG	TCCGCACCAG	GTACTGGTAT
	GGGAGCTCAG	TTTATGCATC	AGCAACGTTC	AGGCGTGGTC	CATGACCATA
9001	CCCACCAAAA	AGTGCGGCGG	CGGCTGGCGG	TAGAGGGGCC	AGCGTAGGGT
• • • •	GGGTGGTTTT	TCACGCCGCC	GCCGACCGCC	ATCTCCCCGG	TCGCATCCCA
9051	GCCCGGGCT	CCGGGGGGGA	GATCTTCCAA	CATAAGGCGA	TGATATCCGT
3031	CCCCCCCC	GGCCCCCGCT	CTAGAAGGTT	GTATTCCGCT	ACTATAGGCA
	CCGGCCCCdv.	000000000			
0101	* C > T C T T C C T	CCACATCCAG	GTGATGCCGG	CGGCGGTGGT	GGAGGCGCGC
9101	AGAIGIACCI	CCCCTTACCTC	CACTACGGCC	GCCGCCACCA	CCTCCGCGCG
	TCTACATGGA	CCIGINGGIC	CACIACOUT		
		50100000000	CCACATCTTC	CCCACCCCCA	AAAAGTGCTC
9151	GGAAAGTCGC	GGACGCGG11	CCAGAIGIIG	CCCTCCCCCT	TTTTCACGAG
	CCTTTCAGCG	CCTGCGCCAA	GGICIACAAC	GCG1CGCCG1	11110
					ייייים ארכרידריד.
9201	CATGGTCGGG	ACCCTCTGGC	CGGTCAGGCG	CGCGCWVICG	TTGACGCTCT
	GTACCAGCCC	TGCGAGACCG	GCCAGTCCGC	GUGUGTIAGU	AACTGCGAGA
9251	AGACCGTGCA	AAAGGAGAGC	CTGTAAGCGG	GCACTCTTCC	GTGGTCTGGT
	TCTGGCACGT	TTTCCTCTCG	GACATTCGCC	CGTGAGAAGG	CACCAGACCA
9301	GGATAAATTC	GCAAGGGTAT	CATGGCGGAC	GACCGGGGTT	CGAGCCCCGT
	CCTATTTAAC	CGTTCCCATA	GTACCGCCTG	CTGGCCCCA	GCTCGGGGCA
9251	אַדררפפררפיז	CCGCCGTGAT	CCATGCGGTT	ACCGCCCGCC	TGTCGAACCC
2221	TAGGCCGC	GGCGGCACTA	GGTACGCCA	A TGGCGGGCGC	ACAGCTTGGG
	TWOOFFGGG				

Figure 27J

9451	CCGCGCCGC	CTGCTGCGCT GACGACGCGA			
	TAAGCGGTTA ATTCGCCAAT				
9551	CCGGAGGGTT GGCCTCCCAA	ATTTTCCAAG TAAAAGGTTC			
9601		CGGACTGCGG GCCTGACGCC			
9651		GCAAATTCCT CGTTTAAGGA			
9701		GCATCCGGTG CGTAGGCCAC			
9751		AAGAGCAGCG TTCTCGTCGC			
9801		GGAGGGGCGA CCTCCCCGCT			
9851	,	CCCGCGCGCGC			
9901		TGGCGCGGCT ACCGCGCCGA			
9951		AAGCGTGATA TTCGCACTAT			
10001		CCGCGAGGGA GGCGCTCCCT			
10051		GGCGCGAGCT CCGCGCTCGA			
10101		GACTTTGAGC CTGAAACTCG			
10151	GCGCACACGT CGCGTGTGCA	CCCCCCCCC	GACCTGGTAA CTGGACCATT	CCGCATACGA GGCGTATGCT	GCAGACGGTG CGTCTGCCAC
10201	AACCAGGAGA TTGGTCCTCT	TTAACTTTCA AATTGAAAGT	AAAAAGCTTT TTTTTCGAAA	AACAACCACG TTGTTGGTGC	TGCGTACGCT ACGCATGCGA
10251	TGTGGCGCGC ACACCGCGCG	GAGGAGGTGG CTCCTCCACC	CTATAGGACT GATATCCTGA	GATGCATCTG CTACGTAGAC	TGGGACTTTG ACCCTGAAAC
10301	TAAGCGCGCT ATTCGCGCGA	GGAGCAAAAC CCTCGTTTTG	CCAAATAGCA GGTTTATCGT	AGCCGCTCAT TCGGCGAGTA	GGCGCAGCTG CCGCGTCGAC

Figure 27K

10401	GCTAAACATA	GTAGAGCCCG	AGGGCCGCTG	GCTGCTCGAT	TTGATAAACA
10101	CGATTTGTAT	CATCTCGGGC	TCCCGGCGAC	CGACGAGCTA	AACTAT TIGT
10451	TOCTGOAGAG	CATAGTGGTG	CAGGAGCGCA	GCTTGAGCCT	GGCTGACAAG
10431	AGGACGTCTC	GTATCACCAC	GTCCTCGCGT	CGAACTCGGA	CCGACTGTTC
10501	GTGGCCGCCA	TCAACTATTC	CATGCTTAGC	CTGGGCAAGT	TTTACGCCCG
10301	CACCGGCGGT	AGTTGATAAG	GTACGAATCG	GACCCGTTCA	AAATGCGGGC
10551	CAAGATATAC	CATACCCCTT	ACGTTCCCAT	AGACAAGGAG	GTAAAGATCG
	GTTCTATATG	GTATGGGGAA	TGCAAGGGTA	TCTGTTCCTC	CATTTCTAGC
10601	AGGGGTTCTA	CATGCGCATG	GCGCTGAAGG	TGCTTACCTT	GAGCGACGAC
20002	TCCCCAAGAT	GTACGCGTAC	CGCGACTTCC	ACGAATGGAA	CTCGCTGCTG
10651	CTGGGCGTTT	ATCGCAACGA	GCGCATCCAC	AAGGCCGTGA	GCGTGAGCCG
			CGCGTAGGTG		
10701	GCGGCGCGAG	CTCAGCGACC	GCGAGCTGAT	GCACAGCCTG	CAAAGGGCCC
			CGCTCGACTA		
10751	TGGCTGGCAC	GGGCAGCGGC	GATAGAGAGG	CCGAGTCCTA	CTTTGACGCG
			CTATCTCTCC		
10801	GGCGCTGACC	TGCGCTGGGC	CCCAAGCCGA	CGCGCCCTGG	AGGCAGCTGG
			GGGTTCGGCT		
10851	GGCCGGACCT	GGGCTGGCGG	TGGCACCCGC	GCGCGCTGGC	AACGTCGGCG
			ACCGTGGGCG		
10901	GCGTGGAGGA	ATATGACGAG	GACGATGAGT	ACGAGCCAGA	GGACGCGAG
			CTGCTACTCA		
10951	TACTAAGCGG	TGATGTTTCT	GATCAGATGA	TGCAAGACGC	AACGGACCCG
					TTGCCTGGGC
11001	GCGGTGCGG	CGGCGCTGCA	GAGCCAGCCG	NCCCCCCAAT	ACTCCACGGA TGAGGTGCCT
11051	CGACTGGCG	CAGGTCATG	ACCGCATCAT	CACCCACTGAC	GCGCGCAATC
					CGCGCGTTAG
11101	CTGACGCGT	CCGGCAGCAC	CCGCAGGCCA	MCCCCCTCTC	CGCAATTCTG
					GCGTTAAGAC
11151	GAAGCGGTG	TCCCGGCGC	CGCAAACCCC	, AUGUAUGAGA	A AGGTGCTGGC
	CTTCGCCAC	AGGCCGCG	C GCGTTTGGGC	, recerecte	1 ICCACGACCG
11201	GATCGTAAA	C GCGCTGGCC	G AAAACAGGG	CATCCGGCC	CACGAGGCCG
	CTAGCATTT	G CGCGACCGG	C TTTTGTCCC	; GTAGGCCGG	, crocrecooc
11251	GCCTGGTCT	A CGACGCGCT	G CTTCAGCGC	TGGCTCGTT	A CAACAGCGGC
	CGGACCAGA	T GCTGCGCGA	C GAAGTCGCG	ACCGAGCAA	T GTTGTCGCCG

Figure 27L

11351		GAGCGCGCGC			
		CICGCGCGCG			
11401	CACTAAACGC	CTTCCTGAGT	ACACAGCCCG	CCAACGTGCC	GCGGGGACAG
	GTGATTTGCG	GAAGGACTCA	TGTGTCGGGC	GGTTGCACGG	CGCCCCTGTC
11451		CCAACTTTGT			
	-	GGTTGAAACA			
11501	ACCGCAAAGT	GAGGTGTACC	AGTCTGGGCC	AGACTATTTT	TTCCAGACCA
		CTCCACATGG	•		
11551		CCTGCAGACC			
		GGACGTCTGG			
11601		GGGGGGTGCG			
		CCCCCACGC			
13.651	TAGCTTGCTG	ACGCCCAACT	CGCGCCTGTT	GCTGCTGCTA	ATAGCGCCCT
		TGCGGGTTGA			
11701	TCACGGACAG	TGGCAGCGTG	TCCCGGGACA	CATACCTAGG	TCACTTGCTG
	•	ACCGTCGCAC			
11751	ACACTGTACC	GCGAGGCCAT	AGGTCAGGCG	CATGTGGACG	AGCATACTTT
		CGCTCCGGTA			
11801		ACAAGTGTCA			
	• • • • • • • • • • • • • • • • • • • •	TGTTCACAGT			
11851		AACCCTAAAC			
		TTGGGATTTG			•
11901		ACAGTTTAAA			
		TGTCAAATTT			
11951		GTGAGCCTTA			
		CACTCGGAAT			
12001		CATGACCGCG			
		GTACTGGCGC			
12051	AACCGGCCGT	TTATCAACCG	CCTAATGGAC	TACTIGCATC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
		AATAGTTGGC			
12101	CGTGAACCCC	GAGTATTTCA	CCAATGCCAT	CTTGAACCCG	CACTGGCTAC
		CTCATAAAGT			
12151	CGCCCCCTGG	TTTCTACACC	GGGGGATTCG	AGGTGCCCGA	GGGTAACGAT
		AAAGATGTGG			
12201	GGATTCCTCT	GGGACGACAT	AGACGACAGC	GTGTTTTCCC	CGCAACCGCA
	CCTAAGGAGA	CCCTGCTGTA	TCTGCTGTCG	CACAAAAGGG	GCGTTGGCGT

Figure 27 M

12301	AGGAAAGCTT	CCGCAGGCCA	AGCAGCTTGT TCGTCGAACA	CCGATCTAGG	CGCTGCGGCC GCGACGCCGG
12351	CCGCGGTCAG	ATGCTAGTAG	CCCATTTCCA	AGCTTGATAG	GGTCTCTTAC
	GGCGCCAGTC	TACGATCATC	GGGTAAAGGT	TCGAACTATC	CCAGAGAATG
12401	CACCACTCCC	ACCACCCGCC	CGCGCCTGCT	GGGCGAGGAG	GAGTACCTAA
12401	GTCGTGAGCG	TGGTGGGCGG	GCGCGGACGA	CCCGCTCCTC	CTCATGGATT
		0000000000	CAGCGCGAAA	AAAACCTCCC	TCCGGCATTT
12451	ACAACTCGCT	CONCERCOS	GTCGCGCTTT	TTTTGGACGG	AGGCCGTAAA
			•		
12501	CCCAACAACG	GGATAGAGAG	CCTAGTGGAC	A AGATGAGTA	GATGGAAGAC
	GGGTTGTTGC	CCTATCTCTC	GGATCACCTG	TTCTACTCAT	CTACCTTCTG
12551	CTACGCGCAG	GAGCACAGGG	ACGTGCCAGG	CCCGCGCCCG	CCCACCCGTC
12551	CATGCGCGTC	CTCGTGTCCC	TGCACGGTCC	GGGCGCGGC	GGGTGGGCAG
12601	GTCAAAGGCA	CGACCGTCAG	CGGGGTCTGG	TGTGGGAGGA	CGATGACTCG
	CAGTTTCCGT	GCTGGCAGTC	GCCCCAGACC	ACACCCTCCT	GCTACTGAGC
12651	GCAGACGACA	GCAGCGTCCT	GGATTTGGGA	GGGAGTGGCA	ACCCGTTTGC
	CGTCTGCTGT	CGTCGCAGGA	CCTAAACCCT	CCCTCACCGT	TGGGCAAACG
12701	ここをしてませてほぐ	CCCAGGCTGG	GGAGAATGTT	TTAAAAAAAA	AAAAAGCATG
12/01	CGTGGAAGCG	GGGTCCGACC	CCTCTTACAA	AATTTTTTTT	TTTTTCGTAC
	> mac > > > \max > \max > \max >	****	CAAGGCCATG	GCACCGAGCG	TTGGTTTTCT
12751	ATGUAAAAIA	TTTTTCACTC	GTTCCGGTAC	CGTGGCTCGC	AACCAAAAGA
	INCGITITAL	1111110000			
12801	TGTATTCCCC	TTAGTATGCG	SCSCSCSCS	ATGTATGAGG	AAGGTCCTCC
12001	ACATAAGGGG	AATCATACGC	CGCGCGCCGC	TACATACTCC	TTCCAGGAGG
12851	TCCCTCCTAC	GAGAGTGTGG	TGAGCGCGGC	GCCAGTGGCG	GCGGCGCTGG
	AGGGAGGATG	CTCTCACACC	ACTCGCGCCG	CGGTCACCGC	CGCCGCGACC
12901	こかかしかしてしてかか	ССАТССТССС	CTGGACCCGC	CGTTTGTGCC	TCCGCGGTAC
12901	CANGAGGGAA	GCTACGAGGG	GACCTGGGCG	GCAAACACGG	AGGCGCCATG
12951	CTGCGGCCTA	CCGGGGGGAG	AAACAGCATC	CGTTACTCTG	AGTTGGCACC
	GACGCCGGAT	GGCCCCCTC	TTTGTCGTAG	GCAATGAGAC	TCAACCGTGG
13001	CCTATTCGAC	ACCACCCGTG	TGTACCTGGT	GGACAACAAG	TCAACGGATG
	GGATAAGCTG	TGGTGGGCAC	ACATGGACCA	CCTGTTGTTC	AGTTGCCTAC
12051	か ただけ 3 かんししむ	GAACTACCAG	AACGACCACA	GCAACTTTCT	GACCACGGTC
13031	1GGCM1CCC1	CTTGATGGTC	TTGCTGGTGT	CGTTGAAAGA	CTGGTGCCAG
	-				
12101	አጥጥር አ አ አ አ ር A	ATGACTACAG	CCCGGGGGAG	GCAAGCACAG	AGACCATCAA
13101	ТААСТТТСТ	TACTGATGTO	GGCCCCCTC	CGTTCGTGT	TCTGGTAGTT
13151	TCTTGACGAC	CGGTCGCACT	GGGGGGGGGG	CCTGAAAAC	ATCCTGCATA
	AGAACTGCTG	GCCAGCGTG	CCCCGCCGCT	GGACTTTTG(TAGGACGTAT

Figure 27N

13251	ССССТСАТСС	TGTCGCGCTT	GCCTACTAAG	GACAATCAGG	TGGAGCTGAA
10232		ACAGCGCGAA			
13301		GTGGAGTTCA			
	TATGCTCACC	CACCTCAAGT	GCGACGGGCT	CCCGTTGATG	AGGCTCTGGT
13351		CCTTATGAAC			
		GGAATACTTG			
13401		ACGGGGTTCT			
		TGCCCCAAGA			
13451		AGACTGGGGT			
		TCTGACCCCA			
13501		AAACGAAGCC			
		TTTGCTTCGG			
13551		ACTTCACCCA			
		TGAAGTGGGT			
13601		CCCTTCCAGG			
		GGGAAGGTCC			
13651		CATTCCCGCA			
		GTAAGGGCGT			
13701		ACACCGAACA			
		TGTGGCTTGT			
13751		GGCGCGGAAG			
•		CCGCGCCTTC			
13801	AGCCGGTGGA	GGACATGAAC	GATCATGCCA	TTCGCGGCGA	CACCTTTGCC
		CCTGTACTTG			
13851		AGGAGAAGCG			
		TCCTCTTCGC			
13901	CGCCCCGCT	GCGCAACCCG	AGGTCGAGAA	GCCTCAGAAG	AAACCGGTGA
		CGCGTTGGGC		•	
13951	TCAAACCCCT	GACAGAGGAC	AGCAAGAAAC	GCAGTTACAA	CCTAATAAGC
		CTGTCTCCTG	_		
14001	AATGACAGCA	CCTTCACCCA	GTACCGCAGC	TGGTACCTTG	CATACAACTA
		GGAAGTGGGT			
14051	CGGCGACCCT	CAGACCGGAA	TCCGCTCATG	GACCCTGCTT	TGCACTCCTG
		GTCTGGCCTT			
14101	ACGTAACCTG	CGGCTCGGAG	CAGGTCTACT	GGTCGTTGCC	AGACATGATG
	TGCATTGGAC	GCCGAGCCTC	GTCCAGATGA	CCAGCAACGG	TCTGTACTAC

Tigure 270

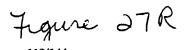
14201	GGTGGGCGCC	GAGCTGTTGC	CCGTGCACTC	CAAGAGCTTC	TACAACGACC
	CCACCCGCGG	CTCGACAACG	GGCACGTGAG	GTTCTCGAAG	ATGTTGCTGG
14251	AGGCCGTCTA				
	TCCGGCAGAT	GAGGGTTGAG	TAGGCGGTCA	AATGGAGAGA	CIGGGIGCAC
14301	ттсаатсест	TTCCCGAGAA	CCAGATTTTG	GCGCGCCGC	CAGCCCCCAC
14301	AAGTTAGCGA	AAGGGCTCTT	GGTCTAAAAC	CGCGCGGGCG	GTCGGGGGTG
14351	CATCACCACC	GTCAGTGAAA	ACGTTCCTGC	TCTCACAGAT	CACGGGACGC
	GTAGTGGTGG	CAGTCACTTT	TGCAAGGACG	AGAGTGTCTA	GIGCCCIGCG
14401	אררכרזכר נ	CAACAGCATC	GGAGGAGTCC	AGCGAGTGAC	CATTACTGAC
14401	ATGGCGACGC	GTTGTCGTAG	CCTCCTCAGG	TCGCTCACTG	GTAATGACTG
			•		
14451	GCCAGACGCC	GCACCTGCCC	CTACGTTTAC	AAGGCCCTGG	GCATAGTCTC
	CGGTCTGCGG	CGTGGACGGG	GATGCAAATG	TTCCGGGACC	CGTATCAGAG
14501	CCCCCCCCTC	CTATCGAGCC	GCACTTTTTG	AGCAAGCATG	TCCATCCTTA
14301	CGGCGCGCAG	GATAGCTCGG	CGTGAAAAAC	TCGTTCGTAC	AGGTAGGAAT
14551	TATCGCCCAG	CAATAACACA	GGCTGGGGCC	TGCGCTTCCC	AAGCAAGATG
	ATAGCGGGTC	GTTATTGTGT	CCGACCCCGG	ACGCGAAGGG	TTCGTTCTAC
14601	mmmcccccc	CCNACNAGCG	CTCCGACCAA	CACCCAGTGC	GCGTGCGCGG
14601	1111666666	CCAAGAAGCG	GAGGCTGGTT	GTGGGTCACG	CGCACGCGCC
14651	GCACTACCGC	GCGCCCTGGG	GCGCGCACAA	ACGCGGCCGC	ACTGGGCGCA
	CGTGATGGCG	CGCGGGACCC	CGCGCGTGTT	TGCGCCGGCG	TGACCCGCGT
		man cocci mc	GACGCGGTGG	TCCACCACCC	CCCCAACTAC
14701	CCACCGICGA	ACTGCGGTAG	CTGCGCCACC	ACCTCCTCCG	CGCGTTGATG
	GGIGGEAGEI	ACTOCOGIAG	C1000000		
14751	ACGCCCACGC	CGCCACCAGT	GTCCACAGTG	GACGCGGCCA	TTCAGACCGT
	TGCGGGTGCG	GCGGTGGTCA	CAGGTGTCAC	CTGCGCCGGT	AAGTCTGGCA
			>mocm>>>>	CANCACACGG	CGGAGGCGCG
14801	GGTGCGCGGA	CCCCCCCCC	TACGATTTTA	CTTCTCTGCC	CCTCCGCGC
	CCACGCGCC1	CGGGCCGCGA	Inconiiiin		
14851	TAGCACGTCG	CCACCGCCGC	CGACCCGGCA	CTGCCGCCCA	ACGCGCGGCG
	ATCGTGCAGC	GGTGGCGGCG	GCTGGGCCGT	GACGGCGGGT	TGCGCGCCGC
				5555555555	
14901	GCGGCCCTGC	TTAACCGCGC	ACGTCGCACC	CCCCCTCCCC	CGGCCATGCG GCCGGTACGC
	CGCCGGGACG	AATTGGCGCG	1GCMGCG1GG	CCGGC1GCCC	00000111000
14951	GGCCGCTCGA	AGGCTGGCCG	CGGGTATTGT	CACTGTGCCC	CCCAGGTCCA
14551	CCGGCGAGCT	TCCGACCGGC	GCCCATAACA	GTGACACGGG	GGGTCCAGGT
15001	GGCGACGAGC	GGCCGCCGCA	GCAGCCGCGG	CCATTAGTGC	TATGACTCAG
	CCGCTGCTCG	CCGGCGGCGT	CGTCGGCGCC	GGTAATCACG	ATACTGAGTC
35051		CC ACCTCTA	"TGGGTGCGC	GACTCGGTTA	GCGGCCTGCG
12021	CCACCACCC	CGTTGCACAT	AACCCACGCG	CTGAGCCAAT	CGCCGGACGC
	CCAGCGICCC				

Figure 27P

15151	ACTTAGACTC	GTACTGTTGT	ATGTATCCAG	CCCCCCCCC	GCGCAACGAA
	TGAATCTGAG	CATGACAACA	TACATAGGTC	GCCGCCGCCG	CGCGTTGCTT
15201	GCTATGTCCA	AGCGCAAAAT	CAAAGAAGAG	ATGCTCCAGG	TCATCGCGCC
25502	CGATACAGGT	TCGCGTTTTA	GTTTCTTCTC	TACGAGGTCC	AGTAGCGCGG
15251	GGAGATCTAT	GGCCCCCGA	AGAAGGAAGA	GCAGGATTAC	AAGCCCCGAA
	CCTCTAGATA	CCGGGGGGCT	TCTTCCTTCT	CGTCCTAATG	TTCGGGGCTT
15301	AGCTAAAGCG	GGTCAAAAAG	AAAAAGAAAG	ATGATGATGA	TGAACTTGAC
	TCGATTTCGC	CCAGTTTTTC	TTTTTCTTTC	TACTACTACT	ACTTGAACTG
15351	GACGAGGTGG	AACTGCTGCA	CGCTACCGCG	CCCAGGCGAC	GGGTACAGTG
2000	•	•		GGGTCCGCTG	
15401	GAAAGGTCGA	CGCGTAAAAC	GTGTTTTGCG	ACCCGGCACC	ACCGTAGTCT
	CTTTCCAGCT	GCGCATTTTG	CACAAAACGC	TGGGCCGTGG	TGGCATCAGA
15451	TTACGCCCGG	TGAGCGCTCC	ACCCGCACCT	ACAAGCGCGT	GTATGATGAG
				TGTTCGCGCA	
15501	GTGTACGGCG	ACGAGGACCT	GCTTGAGCAG	GCCAACGAGC	GCCTCGGGGA
	CACATGCCGC	TGCTCCTGGA	CGAACTCGTC	CGGTTGCTCG	CGGAGCCCCT
15551	GTTTGCCTAC	GGAAAGCGGC	ATAAGGACAT	GCTGGCGTTG	CCGCTGGACG
	CAAACGGATG	CCTTTCGCCG	TATTCCTGTA	CGACCGCAAC	GGCGACCTGC
15601	AGGGCAACCC	AACACCTAGC	CTAAAGCCCG	TAACACTGCA	GCAGGTGCTG
				ATTGTGACGT	
15651	CCCGCGCTTG	CACCGTCCGA	AGAAAAGCGC	GGCCTAAAGC	GCGAGTCTGG
				CCGGATTTCG	•
15701	TGACTTGGCA	CCCACCGTGC	AGCTGATGGT	ACCCAAGCGC	CAGCGACTGG
				TGGGTTCGCG	
15751	AAGATGTCTT	GGAAAAAATG	ACCGTGGAAC	CTGGGCTGGA	GCCCGAGGTC
				GACCCGACCT	
15801	CGCGTGCGGC	CAATCAAGCA	GGTGGCGCCG	GGACTGGGCG	TGCAGACCGT
				CCTGACCCGC	
15851	GGACGTTCAG	ATACCCACTA	CCAGTAGCAC	CAGTATTGCC	ACCGCCACAG
	CCTGCAAGTC	TATGGGTGAT	GGTCATCGTG	GTCATAACGG	TGGCGGTGTC
15901	AGGGCATGGA	GACACAAACG	TCCCCGGTTG	CCTCAGCGGT	GGCGGATGCC
	TCCCGTACCT	CTGTGTTTGC	AGGGGCCAAC	GGAGTCGCCA	CCGCCTACGG
15951	GCGGTGCAGG	CGGTCGCTGC	GCCGCGTCC	AAGACCTCTA	CGGAGGTGCA
	CGCCACGTCC	GCCAGCGACG	CCGGCGCAGG	TTCTGGAGAT	GCCTCCACGT
16001	AACGGACCCG	TGGATGTTTC	GCGTTTCAGC	CCCCCGCGC	CCGCGCCGTT
4000	TTGCCTGGGC	ACCTACAAAG	CGCAAAGTCG	GGGGCCGCG	GGCGCGGCAA

Figure 270

16051	CGAGGAAGTA GCTCCTTCAT	GCCGCGCCGCC CGGCGCCGCC	AGCGCGCTAC TCGCGCGATG	TGCCCGAATA ACGGGCTTAT	TGCCCTACAT ACGGGATGTA
16101	CCTTCCATTG	CGCCTACCCC	CGGCTATCGT	GGCTACACCT	ACCGCCCCAG
19101	GGAAGGTAAC	GCGGATGGGG	GCCGATAGCA	CCGATGTGGA	TGGCGGGGTC
16151	AAGACGAGCA	ACTACCCGAC	GCCGAACCAC	CACTGGAACC	CGCCGCCGCC
10151	TTCTGCTCGT	TGATGGGCTG	CGGCTTGGTG	GTGACCTTGG	GCGGCGGCGG
16201	GTCGCCGTCG	CCAGCCCGTG	CTGGCCCCGA	TTTCCGTGCG	CAGGGTGGCT
	CAGCGGCAGC	GGTCGGGCAC	GACCGGGGCT	AAAGGCACGC	GTCCCACCGA
16251	CGCGAAGGAG	GCAGGACCCT	GGTGCTGCCA	ACAGCGCGCT	ACCACCCCAG
			CCACGACGGT		
16301	CATCGTTTAA	AAGCCGGTCT	TTGTGGTTCT	TGCAGATATG	GCCCTCACCT
			AACACCAAGA		•
16351	GCCGCCTCCG	TTTCCCGGTG	CCGGGATTCC	GAGGAAGAAT	CCTCCCATCC
			GGCCCTAAGG		
16401	AGGGGCATGG	CCGGCCACGG	CCTGACGGGC	CCCTACCCAC	GTGCGCACCA CACGCGTGGT
	TCCCCGTACC	GGCCGGTGCC	GGACTGCCCG	CCGIACGCAG	C C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.
16451	cceccecee	CGCGCGTCGC	ACCGTCGCAT	GCGCGGCGGT	ATCCTGCCCC
10427	GGCCGCCGCC	GCGCGCAGCG	TGGCAGCGTA	CGCGCCGCCA	TAGGACGGGG
16501	TCCTTATTCC	ACTGATCGCC	GCGGCGATTG	GCGCCGTGCC	CGGAATTGCA
1030-	AGGAATAAGG	TGACTAGCGG	CGCCGCTAAC	CGCGGCACGG	GCCTTAACGT
16551	TCCGTGGCCT	TGCAGGCGCA	GAGACACTGA	TTAAAAACAA	GTTGCATGTG
					CAACGTACAC
16601	GAAAAATCAA	AATAAAAAGT	CTGGACTCTC	ACGCTCGCTT	GGTCCTGTAA
					CCAGGACATT
16651	CTATTTTGTA	GAATGGAAGA	CATCAACTTI	GCGTCTCTGG	CCCCGCGACA
	GATAAAACAT	CTTACCTTCI	CTAGTTGAAA	CGCAGAGACC	GGGGCGCTGT
16701	CCCCTCCCC	CCGTTCATGG	GAAACTGGCA	AGATATCGG	ACCAGCAATA
16701	GCCGAGCGCG	GGCAAGTAC	CTTTGACCGI	TCTATAGCC(G TGGTCGTTAT
16751	TGAGCGGTGG	CGCCTTCAG	TGGGGCTCGC	TGTGGAGCG	CATTAAAAAT
	ACTCGCCACC	GCGGAAGTC	ACCCCGAGC	ACACCTCGC	GTAATTTTIA
16801	TTCGGTTCCA	CCGTTAAGA	CTATGGCAGO	AAGGCCTGG	ACAGCAGCAC
	AAGCCAAGGT	GGCAATTCT	CATACCGTC	TTCCGGACC:	r TGTCGTCGTG
16851	AGGCCAGATO	CTGAGGGAT	A AGTTGAAAGI	A GCAAAATTT	CAACAAAAGG
	TCCGGTCTAC	GACTCCCTA'	r TCAACTTTC	r CGTTTTAAA	g Grigiffice
16901	TGGTAGATG	CCTGGCCTC	r GGCATTAGC	G GGGTGGTGG.	A CCTGGCCAAC
	ACCATCTAC	GGACCGGAG	A CCGTAATCG	C CCCACCACC	T GGACCGGTTG
16951	CAGGCAGTG	AAAATAAGA	T TAACAGTAA	G CTTGATCCC	C GCCCTCCCGT
	GTCCGTCAC	G TTTTATTCT.	A ATTGTCATT	C GAACTAGGG	G CGGGAGGGCA



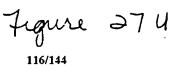
17051				CTCTGGTGAC GAGACCACTG	
17101				CAAGGCCTGC GTTCCGGACG	
17151				GGGCCAGCAC CCCGGTCGTG	
17201				AGCAGAAACC TCGTCTTTGG	
17251				AGCCGCGCGT TCGGCGCGCA	
17301				CGTAGCCAGT GCATCGGTCA	
17351				GGGTGCAATC CCCACGTTAG	
17401				ATGTGTGTCA TACACACAGT	
17451				CCGCGCGCCC	
17501				TCTTACATGC AGAATGTACG	
17551				GCTGGTGCAG CGACCACGTC	
17601				AGTTTAGAAA TCAAATCTTT	
17651	CGCGGATGCG	TGCTGCACTG	GTGTCTGGCC	TCCCAGCGTT AGGGTCGCAA	ACTGCGACGC
17701	GTTCATCCCT CAAGTAGGGA	GTGGACCGTG CACCTGGCAC	AGGATACTGC TCCTATGACG	GTACTCGTAC CATGAGCATG	AAGGCGCGGT TTCCGCGCCA
17751	TCACCCTAGC AGTGGGATCG	TGTGGGTGAT ACACCCACTA	AACCGTGTGC TTGGCACACG	TGGACATGGC ACCTGTACCG	TTCCACGTAC AAGGTGCATG
17801	TTTGACATCC AAACTGTAGG	GCGGCGTGCT CGCCGCACGA	GGACAGGGGC CCTGTCCCCG	CCTACTTTTA GGATGAAAAT	AGCCCTACTC TCGGGATGAG
	TGGCACTGCC ACCGTGACGG				
17901	AATGGGATGA TTACCCTACT	AGCTGCTACT TCGACGATGA	GCTCTTGAAA CGAGAACTTT	TAAACCTAGA ATTTGGATCT	AGAAGAGGAC TCTTCTCCTG

Figure 275

0 02.00					
17951	GATGACAACG	AAGACGAAGT	AGACGAGCAA	GCTGAGCAGC	AAAAAACTCA
	CTACTGTTGC	TTCTGCTTCA	TCTGCTCGTT	CGACTCGTCG	TTTTTTGAGT
18001	CGTATTTGGG	CAGGCGCCTT	ATTCTGGTAT	AAATATTACA	AAGGAGGGTA
	GCATAAACCC	GTCCGCGGAA	TAAGACCATA	TTTATAATGT	TTCCTCCCAT
18051	TTCAAATAGG	TGTCGAAGGT	CAAACACCTA	AATATGCCGA	TAAAACA1111
	AAGTTTATCC	ACAGCTTCCA	GTTTGTGGAT	TTATACGGCT	ATTTTGTAAA
				maam> cc> > >	CACAAATTAA
18101	CAACCTGAAC	CTCAAATAGG	AGAATCTCAG	TGGTACGAAA	CTCTTTAATT
	GTTGGACTTG	GAGTTTATCC	TCTTAGAGTC	ACCATGCTTT	0.0
		GOCA CA CTCC	mananancac	TACCCCAATG	AAACCATGTT
18151	TCATGCAGCT	CCCTCTCAGG	ATTTTTCTG	ATGGGGTTAC	TTTGGTACAA
	AGTACGTCGA	CCCTCTCAGG	A		
18201	አሮርርጥጥር ልጥል	TGCAAAACCC	ACAAATGAAA	ATGGAGGGCA	AGGCATTCTT
10201	TCCCAAGTAT	ACGTTTTGGG	TGTTTACTTT	TACCTCCCGT	TCCGTAAGAA
18251	GTAAAGCAAC	AAAATGGAAA	GCTAGAAAGT	CAAGTGGAAA	TGCAATTTTT
20200	CATTTCGTTG	TTTTACCTTT	CGATCTTTCA	GTTCACCTTT	ACGTTAAAAA
					» CMCCTN » » C
18301	CTCAACTACT	GAGGCAGCCG	CAGGCAATGG	TGATAACTTG	TGAGGATTTC
	GAGTTGATGA	CTCCGTCGGC	GTCCGTTACC	ACTATTGAAC	IGAGGAIIIC
		~. ~~~\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \		AAACCCCAGA	CACTCATATT
18351	TGGTATTGTA	CAGTGAAGAI	CIACATATATA	TTTGGGGTCT	GTGAGTATAA
	ACCATAACAT	GICACIICIA	CAICIAIAIC		
	መርመጥእሮእጥርር	ССАСТАТТАА	GGAAGGTAAC	TCACGAGAAC	TAATGGGCCA
18401	ACADTCTACE	GGTGATAATI	CCTTCCATTC	AGTGCTCTTG	ATTACCCGGT
18451	ACAATCTATG	CCCAACAGGC	CTAATTACAT	TGCTTTTAGG	GACAATTTTA
10431	TGTTAGATAC	GGGTTGTCCG	GATTAATGT	ACGAAAATCO	CTGTTAAAAT
18501	TTGGTCTAAT	GTATTACAAC	AGCACGGGT	A ATATGGGTGT	TCTGGCGGGC
	AACCAGATTA	A CATAATGTTC	TCGTGCCCA:	r TATACCCACA	AGACCGCCCG
				T TTCCAAGAC	GAAACACAGA
18551	CAAGCATCGC	AGTTGAATGC	, IGIIGIAGA RENDENTET	A AACGTTCTG	CTTTGTGTCT
	GTTCGTAGCC	TCAACTIAC	, ACMCAICI		
10601	<u> </u>	CAGCTTTTG	TTGATTCCA	T TGGTGATAG	A ACCAGGTACT
18601	CCAAACTAT(GTCGAAAAC	AACTAAGGT	A ACCACTATO	TGGTCCATGA
18651	TTTCTATGT(GAATCAGGC	r gttgacagc	T ATGATCCAG	A TGTTAGAATT
	AAAGATACA(CTTAGTCCG	A CAACTGTCG	A TACTAGGTC	T ACAATCTTAA
					* CC***********
18701	ATTGAAAAT	C ATGGAACTG	A AGATGAACT	J. CCWWWIIWC	T GCTTTCCACT
	TAACTTTTA	G TACCTTGAC	T TCTACTIGA	M GGIIIMAIG	A CGAAAGGTGA
			ר אכארייריידיא	C CAAGGTAAA	A CCTAAAACAG
18751	GGGAGGTGT	G ATTAATACA C maammamon	C TOTOLOGY TERSESSES	G GTTCCATTT	T GGATTTTGTC
	CMC > CC > > >	A TOGATOGGA	A AAAGATGCT	A CAGAATTT	C AGATAAAAAT
18801	CACACCAAA	T ACCTACCCT	T TTTCTACGA	T GTCTTAAAA	G TCTATTTTA
18851	GAAATAAGA	G TTGGAAATA	A TTTTGCCAT	rg gaaatcaat	C TAAATGCCAA
	CTTTATTCT	C AACCTTTAT	T AAAACGGTA	AC CTTTAGTTA	G ATTTACGGTT

Figure 27T

18951	AGCTAAAGTA TCGATTTCAT	CAGTCCTTCC GTCAGGAAGG	AACGTAAAAA TTGCATTTTT	TTTCTGATAA AAAGACTATT	CCCAAACACC GGGTTTGTGG
19001		TGAACAAGCG ACTTGTTCGC			
19051	CATTAACCTT	GGAGCACGCT CCTCGTGCGA	GGTCCCTTGA CCAGGGAACT	CTATATGGAC GATATACCTG	AACGTCAACC TTGCAGTTGG
19101	CATTTAACCA	CCACCGCAAT GGTGGCGTTA	GCTGGCCTGC	GCTACCGCTC	AATGTTGCTG
19151	GGCAATGGTC	GCTATGTGCC CGATACACGG	CTTCCACATC	CAGGTGCCTC	AGAAGTTCTT
19201	TGCCATTAAA	AACCTCCTTC TTGGAGGAAG	TCCTGCCGGG	CTCATACACC	TACGAGTGGA
19251	ACTTCAGGAA	GGATGTTAAC CCTACAATTG	ATGGTTCTGC	AGAGCTCCCT	AGGAAATGAC
19301	CTAAGGGTTG	ACGGAGCCAG TGCCTCGGTC	CATTAAGTTT	GATAGCATTT	GCCTTTACGC
19351	CACCTTCTTC	CCCATGGCCC GGGTACCGGG	ACAACACCGC	CTCCACGCTT	GAGGCCATGC
19401	TTAGAAACGA	CACCAACGAC GTGGTTGCTG	CAGTCCTTTA	ACGACTATCT	CTCCGCCGCC
19451	AACATGCTCT	ACCCTATACC TGGGATATGG	CGCCAACGCT	ACCAACGTGC	CCATATCCAT
19501	CCCCTCCCGC	AACTGGGCGG TTGACCCGCC	CTTTCCGCGG	CTGGGCCTTC	ACGCGCCTTA
19551	AGACTAAGGA	AACCCCATCA	CTGGGCTCGG	GCTACGACCC	TTATTACACC
19601	TACTCTGGCT	TTGGGGTAGT CTATACCCTA	CCTAGATGGA	ACCTTTTACC	TCAACCACAC
19651	CTTTAAGAAG	GATATGGGAT GTGGCCATTA	CCTTTGACTC	TTCTGTCAGC	TGGCCTGGCA
19701	ATGACCGCCT	CACCGGTAAT GCTTACCCCC	AACGAGTTTG	AAATTAAGCG	CTCAGTTGAC
19751	CCCCACCCTT	CGAATGGGGG ACAACGTTGC	CCAGTGTAAC	ATGACCAAAG	ACTGGTTCCT
	CCCCTCCCAA	TGTTGCAACG	GGTCACATTG	TACTGGTTTC	TGACCAAGGA
27001	CCATGTTTAC	GATCGATTGA	TATTGTAACC	GATGGTCCCG	AAGATATAGG



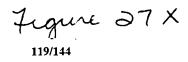
0 02/0220	••				
19851	CAGAGAGCTA GTCTCTCGAT	CAAGGACCGC GTTCCTGGCG	ATGTACTCCT TACATGAGGA	TCTTTAGAAA AGAAATCTTT	CTTCCAGCCC GAAGGTCGGG
19901	ATGAGCCGTC	AGGTGGTGGA	TGATACTAAA	TACAAGGACT	ACCAACAGGT
			ACAACTCTGG		
19951	CCCGTAGGAT	GTGGTTGTGT	TGTTGAGACC	TAÀACAACCG	ATGGAACGGG
20001	CCACCATGCG GGTGGTACGC	CGAAGGACAG GCTTCCTGTC	GCCTACCCTG CGGATGGGAC	CTAACTTCCC GATTGAAGGG	CTATCCGCTT GATAGGCGAA
20051	ATAGGCAAGA TATCCGTTCT	CCGCAGTTGA GGCGTCAACT	CAGCATTACC GTCGTAATGG	CAGAAAAAGT GTCTTTTCA	TTCTTTGCGA AAGAAACGCT
20101	TCGCACCCTT AGCGTGGGAA	TGGCGCATCC ACCGCGTAGG	CATTCTCCAG GTAAGAGGTC	TAACTTTATG ATTGAAATAC	TCCATGGGCG AGGTACCCGC
20151	CACTCACAGA GTGAGTGTCT	CCTGGGCCAA GGACCCGGTT	AACCTTCTCT TTGGAAGAGA	ACGCCAACTC TGCGGTTGAG	CGCCCACGCG GCGGGTGCGC
20201	CTAGACATGA GATCTGTACT	CTTTTGAGGT GAAAACTCCA	GGATCCCATG CCTAGGGTAC	GACGAGCCCA CTGCTCGGGT	CCCTTCTTTA GGGAAGAAAT
20251	TGTTTTGTTT ACAAAACAAA	GAAGTCTTTG CTTCAGAAAC	ACGTGGTCCG TGCACCAGGC	TGTGCACCAG ACACGTGGTC	GGCGTGGCGC
20301	GCGTCATCGA CGCAGTAGCT	AACCGTGTAC TTGGCACATG	CTGCGCACGC GACGCGTGCG	CCTTCTCGGC GGAAGAGCCG	CGGCAACGCC GCCGTTGCGG
20351	ACAACATAAA TGTTGTATTT	GAAGCAAGCA CTTCGTTCGT	ACATCAACAA TGTAGTTGTT	CAGCTGCCGC GTCGACGGCG	CATGGGCTCC GTACCCGAGG
20401	AGTGAGCAGG TCACTCGTCC	AACTGAAAGC	CATTGTCAAA GTAACAGTTT	GATCTTGGTT CTAGAACCAA	GTGGGCCATA CACCCGGTAT
20451	TTTTTTGGGC AAAAAACCCG	ACCTATGACA TGGATACTG	AGCGCTTTCC TCGCGAAAGG	AGGCTTTGT1 TCCGAAACAA	TCTCCACACA AGAGGTGTGT
20501	AGCTCGCCTG TCGAGCGGAC	CGCCATAGTO	AATACGGCCG TTATGCCGGC	GTCGCGAGAC CAGCGCTCTC	TGGGGGCGTA ACCCCCGCAT
20551	CACTGGATGG GTGACCTACG	CCTTTGCCTC	GAACCCGCAC CTTGGGCGTG	TCAAAAACAT AGTTTTTGTA	GCTACCTCTT CGATGGAGAA
20601	TGAGCCCTTT ACTCGGGAAA	GGCTTTTCT(CCGAAAAGA(ACCAGCGACT TGGTCGCTGA	CAAGCAGGTT	TACCAGTTTG
20651	AGTACGAGT(TCATGCTCA(ACTCCTGCGG TGAGGACGC	CGTAGCGCCA GCATCGCGGT	TTGCTTCTT(AACGAAGAA	CCCCGACCGC GGGGCTGGCG
20701	TGTATAACG(ACATATTGC(TGGAAAAGT ACCTTTCA	C CACCCAAAGO G GTGGGTTTCO	GTACAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	CCAACTCGGC GGTTGAGCCG
20751	CGCCTGTGG. GCGGACACC	A CTATTCTGC T GATAAGACG	T GCATGTTTC A CGTACAAAG	r ccacgcctt A ggtgcggaa	T GCCAACTGGC A CGGTTGACCG

Figure 27 V.

20851	CCCAACTCCA	TGCTCAACAG	TCCCCAGGTA	CAGCCCACCC	TGCGTCGCAA
	GGGTTGAGGT	ACGAGTTGTC	AGGGGTCCAT	GTCGGGTGGG	ACGCAGCGTT
20901	CCAGGAACAG	CTCTACAGCT	TCCTGGAGCG	CCACTCGCCC	TACTTCCGCA
		GAGATGTCGA			
20951	GCCACAGTGC	GCAGATTAGG	AGCGCCACTT	CTTTTTGTCA	CTTGAAAAAC
		CGTCTAATCC			
21001	ATGTAAAAAT	AATGTACTAG	AGACACTTTC	AATAAAGGCA	AATGCTTTTA
		TTACATGATC			
21051	TTTGTACACT	CTCGGGTGAT	TATTTACCCC	CACCCTTGCC	GTCTGCGCCG
		GAGCCCACTA			
21101	TTTAAAAAATC	AAAGGGGTTC	TGCCGCGCAT	CGCTATGCGC	CACTGGCAGG
		TTTCCCCAAG			
21151	GACACGTTGC	GATACTGGTG	TTTAGTGCTC	CACTTAAACT	CAGGCACAAC
		CTATGACCAC			
21201	CATCCGCGGC	AGCTCGGTGA	AGTTTTCACT	CCACAGGCTG	CGCACCATCA
-		TCGAGCCACT			
21251	CCAACGCGTT	TAGCAGGTCG	GGCGCCGATA	TCTTGAAGTC	GCAGTTGGGG
		ATCGTCCAGC			
21301	CCTCCGCCCT	GCGCGCGCGA	GTTGCGATAC	ACAGGGTTGC	AGCACTGGAA
		CGCGCGCGCT			
21351	CACTATCAGC	GCCGGGTGGT	GCACGCTGGC	CAGCACGCTC	TTGTCGGAGA
		CGGCCCACCA			
21401	TCAGATCCGC	GTCCAGGTCC	TCCGCGTTGC	TCAGGGCGAA	CGGAGTCAAC
•		CAGGTCCAGG			
21451	TTTGGTAGCT	GCCTTCCCAA	AAAGGGCGCG	TGCCCAGGCT	TTGAGTTGCA
•		CGGAAGGGTT	•		
21501	CTCGCACCGT	AGTGGCATCA	AAAGGTGACC	GTGCCCGGTC	TGGGCGTTAG
	_	TCACCGTAGT		•	
21551	GATACAGCGC	CTGCATAAAA	GCCTTGATCT	GCTTAAAAGC	CACCTGAGCC
	_				GTGGACTCGG
21601	TTTGCGCCTT	CAGAGAAGAA	CATGCCGCAA	GACTTGCCGG	AAAACTGATT
				•	TTTTGACTAA
21651	GGCCGGACAG	GCCGCGTCGT	GCACGCAGCA	CCTTGCGTCG	GTGTTGGAGA
	CCGGCCTGTC	CGGCGCAGCA	CGTGCGTCGT	GGAACGCAGC	CACAACCTCT
21701	TCTGCACCAC	ATTTCGGCCC	CACCGGTTCT	TCACGATCTT	GGCCTTGCTA
	AGACGTGGTG	TAAAGCCGGG	GTGGCCAAGA	AGTGCTAGAA	CCGGAACGAT

7. gure 27 W

21801				TCCGTGTAGA AGGCACATCT	
21851				ACAACGCGCA TGTTGCGCGT	
21901				GACTGCAGGT CTGACGTCCA	
21951				GTTGCTGGTG CAACGACCAC	
22001				TCTTGCATAC AGAACGTATG	
22051				TTCGCCTTTA AAGCGGAAAT	
22101				AGCCTCCATG TCGGAGGTAC	
22151				TCATCACCGT AGTAGTGGCA	
22201				TGCGTCCGCA ACGCAGGCGT	
22251				TGTGCGCTTA ACACGCGAAT	
22301				AACCCACCAT TTGGGTGGTA	
22351				ATTACCTCTG TAATGGAGAC	
22401				TTTCTTCTTG AAAGAAGAAC	
22451				GGCTGGGTGT CCGACCCACA	
22501	AGCGCGTCTT TCGCGCAGAA	GTGATGAGTC CACTACTCAG	TTCCTCGTCC AAGGAGCAGG	TCGGACTCGA AGCCTGAGCT	TACGCCGCCT ATGCGGCGGA
22551	CATCCGCTTT GTAGGCGAAA	TTTGGGGGCG AAACCCCCGC	CCCGGGGAGG GGGCCCCTCC	CGGCGGCGAC	GGGGACGGGG
22601	ACGACACGTC TGCTGTGCAG	CTCCATGGTT GAGGTACCAA	GGGGGACGTC CCCCCTGCAG	GCGCCGCACC CGCGGCGTGG	GCGTCCGCGC CGCAGGCGCG
22651	TCGGGGGTGG AGCCCCCACC	TTTCGCGCTG AAAGCGCGAC	CTCCTCTTCC GAGGAGAAGG	CGACTGGCCA GCTGACCGGT	TTTCCTTCTC AAAGGAAGAG



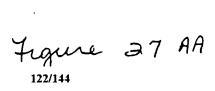
22751	CCGCCCCTC GGCGGGGGAG	TGAGTTCGCC ACTCAAGCGG	ACCACCGCCT TGGTGGCGGA	CCACCGATGC GGTGGCTACG	CGCCAACGCG GCGGTTGCGC
22801				CTTGAGGAGG GAACTCCTCC	
22851				AGACGACGAG TCTGCTGCTC	
22901				ACAACGCAGA TGTTGCGTCT	
22951				GGCGACTACC CCGCTGATGG	
23001	TCTGCTGCAC	GACAACTTCG	TAGACGTCGC	CCAGTGCGCC GGTCACGCGG	TAATAGACGC
23051	ACGCGTTGCA TGCGCAACGT	AGAGCGCAGC TCTCGCGTCG	GATGTGCCCC CTACACGGGG	TCGCCATAGC AGCGGTATCG	GGATGTCAGC CCTACAGTCG
23101				CGCGTACCCC GCGCATGGGG	
23151	AGAAAACGGC TCTTTTGCCG	ACATGCGAGC TGTACGCTCG	CCAACCCGCG GGTTGGGCGC	CCTCAACTTC GGAGTTGAAG	TACCCCGTAT ÄTGGGGCATA
23201	AACGGCACGG	TCTCCACGAA	CGGTGGATAG	ACATCTTTTT TGTAGAAAAA	GGTTTTGACG
23251	TTCTATGGGG	ATAGGACGGC	ACGGTTGGCG	AGCCGAGCGG TCGGCTCGCC	TGTTCGTCGA
23301				TATCGCCTCG ATAGCGGAGC	
23351				ACGAGAAGCG TGCTCTTCGC	
23401	CGAGACGTTG	TCCTTTTGTC	GCTTTTACTT	AGTCACTCTG TCAGTGAGAC	CTCACAACCA
23451	GGAACTCGAG CCTTGAGCTC			CGTACTAAAA GCATGATTTT	
23501	AGGTCACCCA TCCAGTGGGT			ACCTACCCCC TGGATGGGGG	
23551	AGCACAGTCA TCGTGTCAGT			CGTGCGCAGC GCACGCGTCG	
23601	GGATGCAAAT CCTACGTTTA			GGGCCTACCC CCCGGATGGG	

Figure 27 Y

23701	GAGCGACGCA	AACTAATGAT	GGCCGCAGTG	CTCGTTACCG	TGGAGCTTGA
23/01	CTCGCTGCGT	ጥጥር አጥጥ አርጥ አ	CCGCCGTCAC	GAGCAATGGC	ACCTCGAACT
	C100010001	1101111111			
00051	GTGCATGCAG	СССТТСТТТС	CTGACCCGGA	GATGCAGCGC	AAGCTAGAGG
23751	CACGTACGTC	CCCNACAAAC	CACTGGGCCT	CTACGTCGCG	TTCGATCTCC
	CACGIACGIC	GCCAAGAAAC	GAC100000		
	AAACATTGCA	CM1 C1 CCMMM	CCACAGGGCT	ACGTACGCCA	GGCCTGCAAG
23801	TTTGTAACGT	CTACACCTTI	CCMCTCCCCA	TGCATGCGGT	CCGGACGTTC
	TTTGTAACGT	GATGTGGAAA	GC1G1CCCGA	TOCATOCOC.	00000000000
			~>> 0 CMC CMC	かつつかなっつかで	GAATTTTGCA
23851	ATCTCCAACG	TGGAGCTCTG	CAACCIGGIC	ACCAMCCAAC	CTTAAAACGT
•	TAGAGGTTGC	ACCTCGAGAC	GTTGGACCAG	AGGATGGAAC	C117022.001
				mmac > CCCTC	AAGGGCGAGG
23901	CGAAAACCGC	CTTGGGCAAA	ACGTGCTTCA	TTCCACGCTC	MUCCCCCCCC
	GCTTTTGGCG	GAACCCGTTT	TGCACGAAGT	AAGGTGCGAG	Trecedence
					> maam> C> CC
23951	CGCGCCGCGA	CTACGTCCGC	GACTGCGTTT	ACTTATTTCT	ATGCTACACC
	GCGCGGCGCT	GATGCAGGCG	CTGACGCAAA	TGAATAAAGA	TACGATGTGG
24001	TGGCAGACGG	CCATGGGCGT	TTGGCAGCAG	TGCTTGGAGG	AGTGCAACCT
	ACCGTCTGCC	GGTACCCGCA	AACCGTCGTC	ACGAACCTCC	TCACGTTGGA
24051	CAAGGAGCTG	CAGAAACTGC	TAAAGCAAAA	CTTGAAGGAC	CTATGGACGG
24031	CTTCCTCGAC	GTCTTTGACG	ATTTCGTTTT	GAACTTCCTG	GATACCTGCC
24101	CCTTCAACCA	GCGCTCCGTG	GCCGCGCACC	TGGCGGACAT	CATTTTCCCC
24101	CCITCARCOR	CGCGAGGCAC	CGGCGCGTGG	ACCGCCTGTA	GTAAAAGGGG
	GGAAG11GC1	CGCGMCCC			
04151	CANCECEE	TT 2 2 2 CCCT	GCAACAGGGT	CTGCCAGACT	TCACCAGTCA
24151	GAACGCC1GC	A A TOTAL CCC	CGTTGTCCCA	GACGGTCTGA	AGTGGTCAGT
	CTTGCGGACG	AMITITIOGA			
		CA CA A COUTTA	ССУУСТТАТ	CCTAGAGCGC	TCAGGAATCT
24201	AAGCATGTTG	CMGMMC1117	CCTTCAAATA	GGATCTCGCG	AGTCCTTAGA
	TTCGTACAAC	GICTIGAAAI	CCIIGAAAIA		
			こののことであること	· ACTTTGTGCC	CATTAAGTAC
24251	TGCCCGCCAC	CIGCIGIGCA	CITCCIAGCG	TCAAACACGG	GTAATTCATG
	ACGGGCGGTG	GACGACACGI	GAAGGAICGC	, IGAMACACOC	U11212 0 0111 2
				• •• •••••••••••••••••••••••••••••••••	TCCACCTAGC
24301	CGCGAATGCC	CTCCGCCGCT	TIGGGGCCAC	, IGCIACCIIC	TGCAGCTAGC
	GCGCTTACGG	GAGGCGGCGA	AACCCCGGTG	, ACGNIGGANG	ACGTCGATCG
					* ACCCCTGACG
24351	CAACTACCTT	GCCTACCACT	CTGACATAAT	CGAAGACGIG	AGCGGTGACG
	GTTGATGGAA	CGGATGGTG	A GACTGTATTA	A CCTICTGCAC	TCGCCACTGC
24401	GTCTACTGGA	GTGTCACTG	CGCTGCAAC	TATGCACCCC	GCACCGCTCC
	CAGATGACCI	CACAGTGACA	A GCGACGTTG(3 ATACGTGGGG	CGTGGCGAGG
24451	CTGGTTTGCA	ATTCGCAGC	r gcttaacgai	A AGTCAAATTA	TCGGTACCTT
	GACCAAACGT	TAAGCGTCG	A CGAATTGCT	r TCAGTTTAAT	r AGCCATGGAA
24501	TGAGCTGCAG	GCTCCCTCG	CTGACGAAA	A GTCCGCGGC	CCCCCAACT
24301	ACTCGACGTO	CCAGGGAGC	G GACTGCTTT	T CAGGCGCCG	A GGCCCCAACT
DACES	<u>አ</u> አ ር ጥር እ ር ጥር (GGGGCTGTG	G ACGTCGGCT	T ACCTTCGCA	A ATTTGTACCT
24001	WWC 1 CWC 1 C/	CCCCGACAC	TGCAGCCGA	A TGGAAGCGT	T TAAACATGGA
	TIGAGIGAGG	- CCCGACAC			

Figure 27Z

24601	GAGGACTACC	ACGCCCACGA	GATTAGGTTC	TACGAAGACC	AATCCCGCCC
		TGCGGGTGCT			
		• • • • • • • • • • • • • • • • • • • •			
24651	CCCTAATCC	GAGCTTACCG	ССТСССТСАТ	TACCCAGGGC	CACATTCTTG
24031		CTCGAATGGC			
	CGGATTACGC	CICOMIOGC	GGACGCAGIA	A10001CCC0	0.0.72.075.0
24701	CCCNAMMCCN	AGCCATCAAC	*********	እ እ C እ C ጥጥጥ C ጥ	CCTACCAAAC
24701		TCGGTAGTTG			
	CGGTTAACGT	TCGGTAGTTG	1111666666	TICICAAAGA	CGAIGCIIIC
		TTTACTTGGA	000000000000	0000220020	MC N N C C C N N T
24751					
	CCTGCCCCCC	AAATGAACCT	GGGGGTCAGG	CCGCTCCTCG	AGTIGGGTIA
24801		CCGCAGCCCT			
	GGGGGGGGGG	GGCGTCGGGA	TAGTCGTCGT	CGGCGCCCGG	GAACGAAGGG
24851		CCAAAAAGAA			
	TCCTACCGTG	GGTTTTTCTT	CGACGTCGAC	GCCGCCGTG	GGTGCCTGCT
24901		TGGGACAGTC			
	CCTCCTTATG	ACCCTGTCAG	TCCGTCTCCT	CCAAAACCTG	CTCCTCCTCC
24951		GGAAGACTGG			
	TCCTGTACTA	CCTTCTGACC	CTCTCGGATC	TGCTCCTTCG	AAGGCTCCAG
25001	GAAGAGGTGT	CAGACGAAAC	ACCGTCACCC	TCGGTCGCAT	TCCCCTCGCC
		GTCTGCTTTG			
		•		•	
25051	GGCGCCCCAG	AAATCGGCAA	CCGGTTCCAG	CATGGCTACA	ACCTCCGCTC
	CCGCGGGGTC	TTTAGCCGTT	GGCCAAGGTC	GTACCGATGT	TGGAGGCGAG
25101	CTCAGGCGCC	GCCGGCACTG	CCCGTTCGCC	GACCCAACCG	TAGATGGGAC
		CGGCCGTGAC			
	00100000				
25151	ACCACTGGAA	CCAGGGCCGG	TAAGTCCAAG	CAGCCGCCGC	CGTTAGCCCA
23131		GGTCCCGGCC			
	iddidnee				
25201	ACACCAACAA	CAGCGCCAAG	CCTACCCCTC	ATGGCGCGGG	CACAAGAACG
25201		GTCGCGGTTC			
	iciculiui	6100000110	CONTOCCONO	140000000	0.0
25251	001 m1 0mm00	TTGCTTGCAA	CACTOTOCOC	CCAACATCTC	CTTCCCCCC
25251		AACGAACGTT			
	GGTATCAACG	AACGAACGTI	CIGACACCCC	CGIIGIAGAG	dANGC G G G C G
		DOD: 00: DO:	00000000000	mmccccccmx	እርስጥርርጥርር እ
25301	CGCTTTCTTC	TCTACCATCA	CGGCGTGGCC	TACCCCCGIA	MCM1CC1GCA
	GCGAAAGAAG	AGATGGTAGT	GCCGCACCGG	AAGGGGGCAT	TGTAGGACGT
				a> aaaaaaaaa	>000000
25351		CATCTCTACA			
	AATGATGGCA	GTAGAGATGT	CGGGTATGAC	GTGGCCGCCG	TUGUUGTUGT
25401	ACAGCAGCGG	CCACACAGAA	GCAAAGGCGA	CCGGATAGCA	AGACTCTGAC
	TGTCGTCGCC	GGTGTGTCTT	CGTTTCCGCT	GGCCTATCGT	TCTGAGACTG
25451	AAAGCCCAAG	AAATCCACAG	CGGCGGCAGC	AGCAGGAGGA	GGAGCGCTGC
	TTTCGGGTTC	TTTAGGTGTC	GCCGCCGTCG	TCGTCCTCCT	CCTCGCGACG
25501	GTCTGGCGCC	CAACGAACCC	GTATCGACCC	GCGAGCTTAG	AAACAGGATT
	CAGACCGCGG	GTTGCTTGGG	CATAGCTGGG	CGCTCGAATC	TTTGTCCTAA



25551	TTTCCCACTC	TGTATGCTAT	ATTTCAACAG	AGCAGGGGCC	AAGAACAAGA
23334	AAAGGGTGAG	ACATACGATA	TAAAGTTGTC	TCGTCCCCGG	TTCTTGTTCT
25601	GCTGAAAATA	AAAAACAGGT	CTCTGCGATC	CCTCACCCGC	AGCTGCCTGT
	CGACTTTTAT	TTTTTGTCCA	GAGACGCTAG	GGAGTGGGCG	TCGACGGACA
					00000000000
25651	ATCACAAAAG	CGAAGATCAG	CTTCGGCGCA	CGCTGGAAGA	CGCGGAGGCI
	TAGTGTTTTC	GCTTCTAGTC	GAAGCCGCGT	GCGACCTICT	GCGCCICCGA
		AATACTGCGC	ここかごみごかごか ず	AACCACTAGT	TTCGCGCCCT
25701	CTCTTCAGTA	TTATGACGCG	CCACTGAGAA	TTCCTGATCA	AAGCGCGGGA
	GAGAAGICAI	TIMIGACGCS	COMETATION		
25751	TTCAAATT	TAAGCGCGAA	AACTACGTCA	TCTCCAGCGG	CCACACCCGG
23/31	AAGAGTTTAA	ATTCGCGCTT	TTGATGCAGT	AGAGGTCGCC	G GTGTGGGCC
25801	CGCCAGCACC	TGTTGTCAGC	GCCATTATGA	GCAAGGAAAT	TCCCACGCCC
	GCGGTCGTGG	ACAACAGTCG	CGGTAATACT	CGTTCCTTTA	AGGGTGCGGG
25851	TACATGTGGA	GTTACCAGCC	ACAAATGGGA	CTTGCGGCTG	CTCCACCCCA
	ATGTACACCT	CAATGGTCGG	TGTTTACCCT	GAACGCCGAC	CICGACGGGI
		ACCCGAATAA	NCTNCNTCNC	CCCCCCACCC	CACATGATAT
25901	AGACTACTCA	MCCCCGAATAA	TCATCTACTC	CCCCCTGGG	GTGTACTATA
	TCTGATGAGT	1666C11A11	IGHIGINCIC	000000000	
25951	CCCCCCTCAA	CGGAATACGC	GCCCACCGAA	ACCGAATTCT	CCTGGAACAG
25951	GGGCCCAGTT	GCCTTATGCG	CGGGTGGCTT	TGGCTTAAGA	GGACCTTGTC
26001	GCGGCTATTA	CCACCACACC	TCGTAATAAC	CTTAATCCCC	GTAGTTGGCC
	CGCCGATAAT	GGTGGTGTGG	AGCATTATTG	GAATTAGGGG	CATCAACCGG
26051	CGCTGCCCTG	GTGTACCAGG	AAAGTCCCGC	* CCCCCCCCC	GTGGTACTTC
	GCGACGGGAC	CACATGGTCC	TTTCAGGGCG	AGGG1GG1GN	CACCATGAAG
		CONCCCCNN	CTTCACATCA	CTAACTCAGG	GGCGCAGCTT
26101	CCAGAGACGC	CCAGGCCGAA	CANCTOTACT	GATTGAGTCC	CCGCGTCGAA
	GGTCTCTGCG	GGICCGGCII	Craidio		
26151	CCCCCCCCCT	TTCGTCACAG	GGTGCGGTCG	CCCGGGCAGG	GTATAACTCA
20131	CGCCCGCCGA	AAGCAGTGTC	CCACGCCAGC	GGGCCCGTCC	CATATTGAGT
26201	CCTGACAATC	AGAGGGCGAG	GTATTCAGCT	CAACGACGAG	TCGGTGAGCT
	GGACTGTTAG	TCTCCCGCTC	CATAAGTCGA	GTTGCTGCTC	AGCCACTCGA
26251	CCTCGCTTGG	TCTCCGTCCG	GACGGGACAT	* TTCAGATCGC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	GGAGCGAACC	AGAGGCAGGU	CIGCCLIGIA	ANGICIAGE	GCCGCGGCCG
			TOAGGCAATO	CTAACTCTG	AGACCTCGTC
26301	CCCACAACTA	ACTOCCEAGO	AGTCCGTTAC	GATTGAGAC	TCTGGAGCAG
26351	CTCTGAGCCG	CGCTCTGGAG	GCATTGGAA	TCTGCAATT	TOROGRAGIA
	GAGACTCGG	GCGAGACCT	CGTAACCTT	AGACGTTAA	A TAACTCCTCA
26401	TTGTGCCATO	GGTCTACTT	r AACCCCTTC	r CGGGACCTC	CGGCCACTAT
	AACACGGTA(CCAGATGAA	A TTGGGGAAG	A GCCCTGGAG	G GCCGGTGATA
					T CCCCCCACCC
26451	CCGGATCAA	TTATTCCTA	A CTTTGACGC	GIAAAGGAU	T CGGCGGACGG A GCCGCCTGCC
	GGCCTAGTT	A AATAAGGAT	AAACTGUG	- CHILICCIO	A GCCGCCTGCC

Figure 27 AB

26501	CTACGACTGA	ATGTTAAGTG	GAGAGGCAGA	GCAACTGCGC	CTGAAACACC
	GATGCTGACT	TACAATTCAC	CTCTCCGTCT	CGTTGACGCG	GACTTTGTGG
26551	TGGTCCACTG	TCGCCGCCAC	AAGTGCTTTG	CCCGCGACTC	CGGTGAGTTT
	ACCAGGTGAC	AGCGGCGGTG	TTCACGAAAC	GGGCGCTGAG	GCCACTCAAA
26601	TGCTACTTTG	AATTGCCCGA	GGATCATATC	GAGGGCCCGG	CGCACGGCGT
	ACGATGAAAC	TTAACGGGCT	CCTAGTATAG	CTCCCGGGCC	GCGTGCCGCA
26651	CCGGCTTACC	GCCCAGGGAG	AGCTTGCCCG	TAGCCTGATT	CGGGAGTTTA
	GGCCGAATGG	CGGGTCCCTC	TCGAACGGGC	ATCGGACTAA	GCCCTCAAAT
26701	CCCAGCGCCC	CCTGCTAGTT	GAGCGGGACA	GGGGACCCTG	TGTTCTCACT
	GGGTCGCGGG	GGACGATCAA	CTCGCCCTGT	CCCCTGGGAC	ACAAGAGTGA
26751	GTGATTTGCA	ACTGTCCTAA	CCCTGGATTA	CATCAAGATC	TTTGTTGCCA
	CACTAAACGT	TGACAGGATT	GGGACCTAAT	GTAGTTCTAG	AAACAACGGT
26801	TCTCTGTGCT	GAGTATAATA	AATACAGAAA	TTAAAATATA	CTGGGGCTCC
	AGAGACACGA	CTCATATTAT	TTATGTCTTT	AATTTTATAT	GACCCCGAGG
26851	TATCGCCATC	CTGTAAACGC	CACCGTCTTC	ACCCGCCCAA	GCAAACCAAG
	ATAGCGGTAG	GACATTTGCG	GTGGCAGAAG	TGGGCGGGTT	CGTTTGGTTC
26901	GCGAACCTTA	CCTGGTACTT	TTAACATCTC	TCCCTCTGTG	ATTTACAACA
	CGCTTGGAAT	GGACCATGAA	AATTGTAGAG	AGGGAGACAC	TAAATGTTGT
26951	GTTTCAACCC	AGACGGAGTG	AGTCTACGAG	AGAACCTCTC	CGAGCTCAGC
	CAAAGTTGGG	TCTGCCTCAC	TCAGATGCTC	TCTTGGAGAG	GCTCGAGTCG
27001				ACCTGCCGGG TGGACGGCCC	
27051	TGCGTCACCG	GCCGCTGCAC	CACACCTACC	GCCTGACCGT	AAACCAGACT
	ACGCAGTGGC	CGGCGACGTG	GTGTGGATGG	CGGACTGGCA	TTTGGTCTGA
27101	TTTTCCGGAC	AGACCTCAAT	AACTCTGTTT	ACCAGAACAG	GAGGTGAGCT
	AAAAGGCCTG	TCTGGAGTTA	TTGAGACAAA	TGGTCTTGTC	CTCCACTCGA
27151	TAGAAAACCC	TTAGGGTATT	AGGCCAAAGG	CGCAGCTACT	GTGGGGTTTA
	ATCTTTTGGG	AATCCCATAA	TCCGGTTTCC	GCGTCGATGA	CACCCCAAAT
27201	TGAACAATTC	AAGCAACTCT	ACGGGCTATT	CTAATTCAGG	TTTCTCTAGA
	ACTTGTTAAG	TTCGTTGAGA	TGCCCGATAA	GATTAAGTCC	AAAGAGATCT
27251	ATCGGGGTTG	GGGTTATTCT	CTGTCTTGTG	ATTCTCTTTA	TTCTTATACT
	TAGCCCCAAC	CCCAATAAGA	GACAGAACAC	TAAGAGAAAT	AAGAATATGA
27301	AACGCTTCTC	TGCCTAAGGC	TCGCCGCCTG	CTGTGTGCAC	ATTTGCATTT
	TTGCGAAGAG	ACGGATTCCG	AGCGGCGGAC	GACACACGTG	TAAACGTAAA
27351	ATTGTCAGCT	TTTTAAACGC	TGGGGTCGCC	ACCCAAGATG	ATTAGGTACA
	TAACAGTCGA	AAAATTTGCG	ACCCCAGCGG	TGGGTTCTAC	TAATCCATGT
27401	TAATCCTAGG	TTTACTCACC	CTTGCGTCAG	CCCACGGTAC	CACCCAAAAG
	ATTAGGATCC	AAATGAGTGG	GAACGCAGTC	GGGTGCCATG	GTGGGTTTTC

Figure 27 AC

O 02/0220					1 € 1/650.
27451	GTGGATTTTA	AGGAGCCAGC	CTGTAATGTT	ACATTCGCAG	CTGAAGCTAA
	CACCTAAAAT	TCCTCGGTCG	GACATTACAA	TGTAAGCGTC	GACTTCGATT
27501	TCACTCCACC	ACTOTTATAA	AATGCACCAC	AGAACATGAA	AAGCTGCTTA
27501	ACTCACGTGG	TGAGAATATT	TTACGTGGTG	TCTTGTACTT	TTCGACGAAT
27551	דידרכרר אר א א	TTAAAATT	GGCAAGTATG	CTGTTTATGC	TATTTGGCAG
27551	AAGCGGTGTT	TTTGTTTTAA	CCGTTCATAC	GACAAATACG	ATAAACCGTC
27601	CCAGGTGACA	CTACAGAGTA	TAATGTTACA	GTTTTCCAGG	GTAAAAGTCA
2,002	GGTCCACTGT	GATGTCTCAT	ATTACAATGT	CAAAAGGTCC	CATTTTCAGT
27651	TAAAACTTTT	ATGTATACTT	TTCCATTTTA	TGAAATGTGC	GACATTACCA
	ATTTTGAAAA	TACATATGAA	AAGGTAAAAT	ACTTTACACG	CTGTAATGGT
27701	TGTACATGAG	CAAACAGTAT	AAGTTGTGGC	CCCCACAAAA	TTGTGTGGAA
• • • • • • • • • • • • • • • • • • • •	ACATGTACTC	GTTTGTCATA	TTCAACACCG	GGGGTGTTTT	AACACACCTT
27751	AACACTGGCA	CTTTCTGCTG	CACTGCTATG	CTAATTACAG	TGCTCGCTTT
				GATTAATGTC	
27801	GGTCTGTACC	CTACTCTATA	TTAAATACAA	AAGCAGACGC	AGCTTTATTG
				TTCGTCTGCG	
27851	AGGAAAAGAA	AATGCCTTAA	TTTACTAAGT	TACAAAGCTA	ATGTCACCAC
				ATGTTTCGAT	
27901	TAACTGCTTT	ACTCGCTGCT	TGCAAAACAA	ATTCAAAAAG	TTAGCATTAT
					AATCGTAATA
27951	AATTAGAATA	GGATTTAAAC	CCCCCGGTCA	TTTCCTGCTC	AATACCATTC
					TTATGGTAAG
28001	CCCTGAACAA	TTGACTCTAT	GTGGGATATG	CTCCAGCGCT	ACAACCTTGA
					TGTTGGAACT
28051	AGTCAGGCTT	CCTGGATGTC	AGCATUTGAL	TITIGGCCAGC	ACCTGTCCCG TGGACAGGGC
				•	
28101	CGGATTTGTT	CCAGTCCAAC	TACAGCGACC	CACCCTAACA	GAGATGACCA
					CTCTACTGGT
28151	ACACAACCAA	Ceceeccecc	GCTACCGGAC	TTACATCTAC	CACAAATACA
					GTGTTTATGT
28201	CCCCAAGTTT	CTGCCTTTGT	CAATAACTGG	GATAACTTGG	GCATGTGGTG
					CGTACACCAC
28251	GTTCTCCATA	GCGCTTATGT	TTGTATGCCT	PATTATTAT 1	TGGCTCATCT
					ACCGAGTAGA
28301	GCTGCCTAA	GCGCAAACGC	GCCCGACCAC	CCATCTATAC	TCCCATCATT
	CGACGGATTI	CGCGTTTGCC	CGGGCTGGT(GGTAGATAT	AGGGTAGTAA
28351	GTGCTACACC	CAAACAATGA	TGGAATCCA	AGATTGGAC	GACTGAAACA
	CACGATGTG	GTTTGTTACT	ACCTTAGGT	A TCTAACCTG	CTGACTTTGT

Figure 27AD

28451		CTGACCCTTG GACTGGGAAC			
28501		TCACATCGAA AGTGTAGCTT			
28551		GATTTGTCAC CTAAACAGTG			
28601		TTTATCCAGT AAATAGGTCA			
28651		CCATCCCCAG GGTAGGGGTC			
28701		AATTATGAAA TTAATACTTT			
28751		CGTTTTGTTC GCAAAACAAG			
28801		ACTCGTATAT TGAGCATATA			
28851		CGAAGCCTGG GCTTCGGACC			
28901		TCTTAGCCCT AGAATCGGGA			
28951		GATGCCATGA CTACGGTACT			
29001		ACAAGTTGTT TGTTCAACAA			
29051	CGCCCACCTT GCGGGTGGAA	CTCCCACCC GAGGGTGGGG	CACTGAAATC GTGACTTTAG	AGCTACTTTA TCGATGAAAT	ATCTAACAGG TAGATTGTCC
29101		TGACACCCTA ACTGTGGGAT			
29151	CAGCGCCTGC GTCGCGGACG	TAGAAAGACG ATCTTTCTGC	CAGGGCAGCG GTCCCGTCGC	GCCGAGCAAC CGGCTCGTTG	AGCGCATGAA TCGCGTACTT
29201	TCAAGAGCTC AGTTCTCGAG	CAAGACATGG GTTCTGTACC	TTAACTTGCA AATTGAACGT	CCAGTGCAAA GGTCACGTTT	AGGGGTATCT TCCCCATAGA
29251	TTTGTCTCGT AAACAGAGCA	AAAGCAGGCC TTTCGTCCGG	AAAGTCACCT TTTCAGTGGA	ACGACAGTAA TGCTGTCATT	TACCACCGGA ATGGTGGCCT
29301	CACCGCCTTA GTGGCGGAAT	GCTACAAGTT CGATGTTCAA	GCCAACCAAG CGGTTGGTTC	CGTCAGAAAT GCAGTCTTTA	TGGTGGTCAT ACCACCAGTA

Figure 27 A E

		****	CAAGGACCTG	ACCATCTCTC	CACCCጥጥልጥጥ
29401	GCTGCATTCA	CTCACCTIGT	GTTCCTGGAC	TCCTACACAC	GTGGGAATAA
	CGACGTAAGT	GAGTGGAACA	GIICCIGGAC	recramone	0.000.0
20451	* * こ * こ * こ こ こ こ で こ で こ で こ で こ で こ	CCCCTCTCAA	AGATCTTATT	CCCTTTAACT	AATAAAAAA
29451	TTCTCCCACA	CCCCAGAGTT	TCTAGAATAA	GGGAAATTGA	TTATTTTTTT
	TICIGGGACA	CGCCAGAG1.	.010.		
29501	AATAATAAAG	CATCACTTAC	TTAAAATCAG	TTAGCAAATT	TCTGTCCAGT
23301	TTATTATTTC	GTAGTGAATG	AATTTTAGTC	AATCGTTTAA	AGACAGGTCA
	_				
29551	TTATTCAGCA	GCACCTCCTT	GCCCTCCTCC	CAGCTCTGGT	ATTGCAGCTT
	AATAAGTCGT	CGTGGAGGAA	CGGGAGGAGG	GTCGAGACCA	TAACGTCGAA
					mai ommmoom
29601	CCTCCTGGCT	GCAAACTTTC	TCCACAATCT	AAATGGAATG	TCAGTTTCCT
	GGAGGACCGA	CGTTTGAAAG	AGGTGTTAGA	TTTACCTTAC	AGTCAAAGGA
			CCCACTATCT	ጥር አጥር ጥጥር ጥጥ	CCACATGAAG
29651	CCTGTTCCTG	TCCATCCGCA	GGGTGATAGA	ACTACAACAA	CGTCTACTTC
	GGACAAGGAC	AGGTAGGCGT	GGGIGNINGN	AGIACIBICIE:	00200
29701	CCCCCAAGAC	CCTCTGAAGA	TACCTTCAAC	CCCGTGTATC	CATATGACAC
29/01	OTOTTO OTOTO	GCAGACTTCT	ATGGAAGTTG	GGGCACATAG	GTATACTGTG
29751	GGAAACCGGT	CCTCCAACTG	TGCCTTTTCT	TACTCCTCCC	TTTGTATCCC
	CCTTTGGCCA	GGAGGTTGAC	ACGGAAAAGA	ATGAGGAGGG	AAACATAGGG
29801	CCAATGGGTT	TCAAGAGAGT	CCCCTGGGG	TACTCTCTTT	GCGCCTATCC
	GGTTACCCAA	AGTTCTCTCA	GGGGGACCCC	ATGAGAGAAA	CGCGGATAGG
			TGGCATGCTT	CCCCTCAAAA	TOCOCANOG
29851	GAACCTCTAG	TTACCTCCAA	ACCGTACGAA	CCCCACTTT	ACCCGTTGCC
	CTTGGAGATC	AATGGAGGTT	ACCGIACGAA	CGCGAG:	
29901		GACGAGGCCG	GCAACCTTAC	CTCCCAAAAT	GTAACCACTG
23301	GGAGAGAGAC	CTGCTCCGGC	CGTTGGAATG	GAGGGTTTTA	CATTGGTGAC
29951	TGAGCCCACC	TCTCAAAAAA	ACCAAGTCAA	ACATAAACCT	GGAAATATCT
	ACTCGGGTGG	AGAGTTTTTT	TGGTTCAGTT	TGTATTTGGA	CCTTTATAGA
	•				000000000000
30001	GCACCCCTCA	CAGTTACCTC	AGAAGCCCTA	ACTGTGGCTG	CCCCCCCTCC
	CGTGGGGAGT	GTCAATGGAG	TCTTCGGGAT	TGACACCGAC	GGCGGCG1GG
		0000007707	ことでできることが	CCAATCACAG	GCCCCGCTAA
30051	TCTAATGGTC	CCCCCCTTCT	GTGAGTGGTA	CGTTAGTGTC	CGGGGCGATT
	AGATTACCAG	(6(((61161	G1GAG1GG1A	00111110101	
20101	CCGTGCACGA	CTCCAAACTT	AGCATTGCCA	CCCAAGGACC	CCTCACAGTG
30101	GGCACGTGCT	GAGGTTTGAA	TCGTAACGGT	GGGTTCCTGG	GGAGTGTCAC
30151	TCAGAAGGAA	AGCTAGCCCT	GCAAACATCA	GGCCCCCTCA	CCACCACCGA
	AGTCTTCCTT	TCGATCGGGA	CGTTTGTAGT	CCGGGGGAGI	GETGETGECT
30201	TAGCAGTACC	CTTACTATCA	CTGCCTCACC	CCCTCTAACT	ACTGCCACTG
	ATCGTCATGG	GAATGATAGI	GACGGAGTGG	GGGAGATIGA	TGACGGTGAC
			* *******	ያ ጋልባልጥል ተነው ተነው ነው።	ZAATGCAAAA
30251	GTAGCTTGGG	CATTGACTTG	, ЖЖИСИССС И 11 ТОВОТОВОВОВОВОВОВОВОВОВОВОВОВОВОВОВОВОВ	LDTDTATAGAGA	AAATGGAAAA TTTACCTTTT
	CATCGAACCC	GIMACIGAAC	, 111010661		

Ligure 27 AF

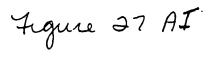
20251	TTTGACCGTA	CC 3 3 CTCCTC	CACCTCTCAC	ТАТТААТААТ	ል ርተጥርርተጥርር
30331					TGAAGGAACG
		•			
30401	AAACTAAAGT				
	TTTGATTTCA	ATGACCTCGG	AACCCAAAAC	TAAGTGTTCC	GTTATACGTT
20451	CTTAATGTAG	CACCACCACT	AACCATTCAT	TCTCAAAACA	GACGCCTTAT
30431					CTGCGGAATA
	CARLE THOMAS				
30501				AAACCAACTA	
	TGAACTACAA	TCAATAGGCA	AACTACGAGT	TTTGGTTGAT	TTAGATTCTG
20554	7. 7. 1. 1. 1. 1. 1. 1. 1. 1		>#>>>0#00>C	CCCACAACTT	CCAMAMMAAC
30551				GGGTGTTGAA	
	AICCIGICCC	GGGAGAAAA	INITIGACIC	9991911924	CCIMINATIO
30601				TCAAACAATT	
	ATGTTGTTTC	CGGAAATGAA	CAAATGTCGA	AGTTTGTTAA	GGTTTTTCGA
				01 mamman 0	GGT1 C1 CCC1
30651				GATGTTTGAC CTACAAACTG	
	ACTCCAATTG	GATTCGTGAC	GGTTCCCCAA	CINCAMETO	CGATGTCGGT
30701	TAGCCATTAA	TGCAGGAGAT	GGGCTTGAAT	TTGGTTCACC	TAATGCACCA
	ATCGGTAATT	ACGTCCTCTA	CCCGAACTTA	AACCAAGTGG	ATTACGTGGT
30751	AACACAAATC			GTACCGGATC	
	TIGIGITIAG	GGGAGIIIIG	IIIIIAACCG	GIACCGGAIC	TIMMCIANG
30801	AAACAAGGCT	ATGGTTCCTA	AACTAGGAAC	TGGCCTTAGT	TTTGACAGCA
	TTTGTTCCGA	TACCAAGGAT	TTGATCCTTG	ACCGGAATCA	AAACTGTCGT
30851				ATGATAAGCT TACTATTCGA	
	GTCCACGGTA	AIGICAICCI	HIGHTHIAL	INCINITION	11GAAACACC
30901	ACCACACCAG	CTCCATCTCC	TAACTGTAGA	CTAAATGCAG	AGAAAGATGC
	TGGTGTGGTC	GAGGTAGAGG	ATTGACATCT	GATTTACGTC	TCTTTCTACG
30951				CAGTCAAATA GTCAGTTTAT	
	ATTIGAGIGA	AACCAGAATT	GITITACACC	GICAGIIIAI	GAACGATGTC
31001	TTTCAGTTTT	GGCTGTTAAA	GGCAGTTTGG	CTCCAATATC	TGGAACAGTT
	AAAGTCAAAA	CCGACAATTT	CCGTCAAACC	GAGGTTATAG	ACCTTGTCAA
					D0000100111
31051	CAAAGTGCTC			CTTTTACCTC	
	GTTTCACGAG	TAGAATAATA	TTCTAAACTG	CITIACCIC	ACGN1GA111
31101	CAATTCCTTC	CTGGACCCAG	AATATTGGAA	CTTTAGAAAT	GGAGATCTTA
	GTTAAGGAAG	GACCTGGGTC	TTATAACCTT	GAAATCTTTA	CCTCTAGAAT
31151	CTGAAGGCAC				
	GACTTCCGTG	TUGGATATGT	TIGUGACAAC	CTAAATACGG	ATTOGATAGT
31201	GCTTATCCAA	AATCTCACGG	TAAAACTGCC	AAAAGTAACA	TTGTCAGTCA
	CGAATAGGTT				

Figure 27 AG

31251	AGTTTACTTA TCAAATGAAT	AACGGAGACA TTGCCTCTGT	AAACTAAACC TTTGATTTGG	TGTAACACTA ACATTGTGAT	ACCATTACAC TGGTAATGTG
	m	ACACCAAACA	GGAGACACAA	CTCCAAGTGC	ATACTCTATG
31301	ATTTGCCATG	TGTCCTTTGT	CCTCTGTGTT	GAGGTTCACG	TATGAGATAC
31351	ጥር አጥጥጥጥር ልጥ	CCCACTCCTC	TGGCCACAAC	TACATTAATG	AAATATTTGC
31351	AGTAAAAGTA	CCCTGACCAG	ACCGGTGTTG	ATGTAATTAC	TTTATAAACG
		ምእ <i>ር</i> እርጥጥጥጥ	CATACATTGC	CCAAGAATAA	AGAATCGTTT
31401	GTGTAGGAGA	ATGTGAAAAA	GTATGTAACG	GGTTCTTATT	TCTTAGCAAA
21451	ርመርመጥ አጥርጥጥ	ጥር እ እ ር ር ጥር ጥጥ	TATTTTTCAA	TTGCAGAAAA	TTTCAAGTCA
31451	CACAATACAA	AGTTGCACAA	ATAAAAAGTT	AACGTCTTTT	AAAGTTCAGT
	CMCMAINCAN	101100110121			
31501	TTTTCATTC	AGTAGTATAG	CCCCACCACC	ACATAGCTTA	TACAGATCAC
31301	AAAAAGTAAG	TCATCATATC	GGGGTGGTGG	TGTATCGAAT	ATGTCTAGTG
31551	ССТАССТТАА	TCAAACTCAC	AGAACCCTAG	TATTCAACCT	GCCACCTCCC
31331	GCATGGAATT	AGTTTGAGTG	TCTTGGGATC	ATAAGTTGGA	CGGTGGAGGG
31601	TCCCAACACA	CAGAGTACAC	AGTCCTTTCT	CCCCGGCTGG	CCTTAAAAAG
31001	AGGGTTGTGT	GTCTCATGTG	TCAGGAAAGA	GGGGCCGACC	GGAATTTTTC
31651	CATCATATCA	TGGGTAACAG	ACATATTCTT	AGGTGTTATA	TTCCACACGG
	GTAGTATAGT	ACCCATTGTC	TGTATAAGAA	TCCACAATAT	AAGGTGTGCC
31701	TTTCCTGTCG	AGCCAAACGC	TCATCAGTGA	TATTAATAAA	CTCCCCGGGC
	AAAGGACAGC	TCGGTTTGCG	AGTAGTCACT	ATAATTATTT	GAGGGGCCCG
31751	AGCTCACTTA	AGTTCATGTC	GCTGTCCAGC	TGCTGAGCCA	CAGGCTGCTG
31,01	TCGAGTGAAT	TCAAGTACAG	CGACAGGTCG	ACGACTCGGT	GTCCGACGAC
31801	TCCAACTTGC	GGTTGCTTAA	CGGGCGCGA	AGGAGAAGTC	CACGCCTACA
32332	AGGTTGAACG	CCAACGAATT	GCCCGCCGCT	TCCTCTTCAG	GTGCGGATGT
31851	TGGGGGTAGA	GTCATAATCG	TGCATCAGGA	TAGGGCGGTG	GTGCTGCAGC
52022	ACCCCCATCT	CAGTATTAGC	ACGTAGTCCT	ATCCCGCCAC	CACGACGTCG
31901	AGCGCGCGAA	TAAACTGCTG	CCGCCGCCGC	TCCGTCCTGC	AGGAATACAA
	TCGCGCGCTT	ATTTGACGAC	GCCGCCGCG	AGGCAGGACG	TCCTTATGTT
31951	CATGGCAGTG	GTCTCCTCAG	CGATGATTCG	CACCGCCCGC	AGCATAAGGC
	GTACCGTCAC	CAGAGGAGTC	GCTACTAAGC	GTGGCGGGCG	TCGTATTCCG
32001	CCCTTCTCCT	CCGGGCACAG	CAGCGCACCC	TGATCTCACT	TAAATCAGCA
	CGGAACAGGA	GGCCCGTGTC	: GTCGCGTGGG	ACTAGAGTGA	ATTTAGTCGT
32051	CAGTAACTGC	AGCACAGCAC	CACAATATTG	TTCAAAATCC	CACAGTGCAA
	GTCATTGACG	TCGTGTCGTG	GTGTTATAAC	AAGTTTTAGG	GTGTCACGTT
32101	GGCGCTGTAT	CCAAAGCTCA	TGGCGGGGAC	CACAGAACCC	ACGTGGCCAT
	CCGCGACATA	GGTTTCGAG1	r Accecccte	GTGTCTTGGG	TGCACCGGTA
22151	CATACCACAZ	GCGCAGGTA	ATTAAGTGG	GACCCCTCAT	AAACACGCTG
J21J1	GTATGGTGTT	CGCGTCCAT	TAATTCACCG	CTGGGGAGT	A TTTGTGCGAC

Figure 27 AH

32251	CCATATAAAC	CTCTGATTAA	ACATGGCGCC	ATCCACCACC	ATCCTAAACC
		GAGACTAATT			
32301		AACCTGCCCG			
		TTGGACGGGC			
32351		AGTGGAGAGC			
		TCACCTCTCG			
32401		TCAATGTTGG			
		AGTTACAACC			
32451		AAGCTCCTCC			
		TTCGAGGAGG	_		
32501		TCAGCGTAAA			
		AGTCGCATTT			
32551		TGCATTGTCA ACGTAACAGT			
	TGAGTGCAAC	ACGTAACAGT	TICACAAIGI	AAGCCCGTCG	TEGECTACIA
32601		GGTAGCGCGG			
		CCATCGCGCC			
32651	CTACTGTACG	GAGTGCGCCG	AGACAACCGA	GATCGTGTTG	GTCGTAGTGT
	•	CTCACGCGGC			
32701		GGAACGCCGG			
	•	CCTTGCGGCC			
32751		GACAAACAGA			
		CTGTTTGTCT			
32801		TAGTTGTAGT			
		ATCAACATCA			
32851		GGGTTCTATG			
	GGGACCGAAG	CCCAAGATAC	ATTIGAGGAA	GIACGCGGCG	ACGGGACIAI
32901		CCGCAGAATA			
	TGTAGGTGGT	GGCGTCTTAT	TCGGTGTGGG	TCGGTTGGAT	GTGTAAGCAA
32951					ACCATGTTTT
		GTGTGCCCTC			
33001		CCAAAAGATT			
		GGTTTTCTAA			
33051	GTGAACGCGC	TCCCCTCCGG	TGGCGTGGTC	AAACTCTACA	GCCAAAGAAC
		AGGGGAGGCC			·
33101		ATTTGTAAGA			
	TCTATTACCG	TAAACATTCT	ACAACGTGTT	ACCGAAGGTT	TTCCGTTTGC



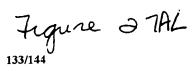
33201	CTCTATAAAC GAGATATTTG	ATTCCAGCAC TAAGGTCGTG	CTTCAACCAT GAAGTTGGTA	GCCCAAATAA CGGGTTTATT	TTCTCATCTC AAGAGTAGAG
33251	GCCACCTTCT CGGTGGAAGA	CAATATATCT GTTATATAGA	CTAAGCAAAT GATTCGTTTA	CCCGAATATT GGGCTTATAA	AAGTCCGGCC TTCAGGCCGG
33301	ATTGTAAAAA TAACATTTT	TCTGCTCCAG AGACGAGGTC	AGCGCCCTCC TCGCGGGAGG	ACCTTCAGCC TGGAAGTCGG	TCAAGCAGCG AGTTCGTCGC
33351	AATCATGATT TTAGTACTAA	GCAAAAATTC CGTTTTTAAG	AGGTTCCTCA TCCAAGGAGT	CAGACCTGTA GTCTGGACAT	TAAGATTCAA ATTCTAAGTT
33401	TTCGCCTTGT	TTAACAAAA AATTGTTTTT	ATGGCGCTAG	GGCATCCAGG	GAAGCGTCCC
33451	GGTCGACTTG	ATAATCGTGC TATTAGCACG	TCCAGACGTG	CCTGGTCGCG	CCGGTGAAGG
33501	GGCGGTCCTT	CCATGACAAA GGTACTGTTT	TCTTGGGTGT	GACTAATACT	GTGCGTATGA
33551	GCCTCGATAC	CTAACCAGCG GATTGGTCGC	ATCGGGGCTA	CATTCGAACA	ACGTACCCGC
33601	CGCTATATTT	ATGCAAGGTG TACGTTCCAC	GACGAGTTTT	TTAGTCCGTT	TCGGAGCGCG
33651	TTTTTTCTTT	GCACATCGTA CGTGTAGCAT	CAGTACGAGT	ACGTCTATTT	CCGTCCATTC
33701	GAGGCCTTGG	ACCACAGAAA TGGTGTCTTT	TTCTGTGGTA	AAAAGAGAGT	TTGTACAGAC
33751	GCCCAAAGAC	GTATTTGTGT	TATTTTATT	TGTTTTTTG	ATTTAAACAT TAAATTTGTA
33801	ATCTTCGGAC	AGAATGTTGT	CCTTTTTGTT	GGGAATATTC	CATAAGACGG GTATTCTGCC
33851	TGATGCCGGT	ACGGCCGCAC	TGGCATTTT	TTGACCAGTG	CGTGATTAAA GCACTAATTT
	TICGIGGIGG	CTGTCGAGGA	GCCAGTACAG	GCCTCAGTAT	ATGTAAGACT TACATTCTGA
	GCCATTTGTG	TAGTCCAACT	AAGTGTAGCC	AGTCACGATT	AAAGCGACCG TTTCGCTGGC
	TTTATCGGGC	CCCCTTATGT	ATGGGCGTCC	GCATCTCTGT	ACATTACAGC TGTAATGTCG
34051	CCCCATAGGA GGGGTATCCT	GGTATAACAA CCATATTGTT	AATTAATAGO TTAATTATCO	AGAGAAAAA(TCTCTTTTT(ACATAAACAC G TGTATTTGTG

Figure 27AJ

34151		CTTCCACAGC GAAGGTGTCG			
34201	AAAAGAAAAC TTTTCTTTTG	CTATTAAAAA GATAATTTTT			
34251		TAAAAAAGGG ATTTTTTCCC			
34301	TTTTTACTGC	TAACGGTTAA ATTGCCAATT	TCAGGTGTTT	TTTGTGGGTC	TTTTGGCGTG
34351	CGCTTGGATG	GCCCAGAAAC CGGGTCTTTG	CTTTCGGTTT	TTTGGGTGTT	GAAGGAGTTT
34401	AGCAGTGAAG	CGTTTTCCCA GCAAAAGGGT	GCAATGCAGT	GAAGGGTAAA	ATTCTTTTGA
34451	TGTTAAGGGT	ACACATACAA TGTGTATGTT	CAATGAGGCG	GGATTTTGGA	TGCAGTGGGC
34501		ACGCCCCGCG TGCGGGGCGC			GGAGTAATAG
					PacI
34551	ATATTGGCTT TATAACCGAA	CAATCCAAAA GTTAGGTTTT			
	AATTCGGATC TTAAGCCTAG				
34651	GAGCGAAGGC	GCGGCATCGG CGCCGTAGCC	CTACGGGCGC	AACGTCCGGT	ACGACAGGTC
34701	CGTCCATCTA	GACGACCATC CTGCTGGTAG	TCCCTGTCGA	AGTTCCGGTC	GTTTTCCGGT
34751	GGAACCGTAA CCTTGGCATT	AAAGGCCGCG TTTCCGGCGC			
		ATCACAAAAA TAGTGTTTTT	TCGACGCTCA AGCTGCGAGT	AGTCAGAGGT TCAGTCTCCA	GGCGAAACCC CCGCTTTGGG
34851	GGACTGCTCG GACAGGACTA CTGTCCTGAT	ATCACAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG	TCGACGCTCA AGCTGCGAGT AGGCGTTTCC TCCGCAAAGG	AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG	GGCGAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG
34851 34901	GGACTGCTCG GACAGGACTA CTGTCCTGAT GCTCTCCTGT CGAGAGGACA	ATCACAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGGAC	TCGACGCTCA AGCTGCGAGT AGGCGTTTCC TCCGCAAAGG CCGCTTACCG GGCGAATGGC	AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG	GGCGAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG CGCCTTTCTC GCGGAAAGAG
34851 34901 34951	GGACTGCTCG GACAGGACTA CTGTCCTGAT GCTCTCCTGT CGAGAGGACA CCTTCGGGAA	ATCACAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGGAC GCGTGGCGCT CGCACCGCGA	TCGACGCTCA AGCTGCGAGT AGGCGTTTCC TCCGCAAAGG CCGCTTACCG GGCGAATGGC TTCTCATAGC AAGAGTATCG	AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG TCACGCTGTA AGTGCGACAT	GGCGAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG CGCCTTTCTC GCGGAAAGAG GGTATCTCAG CCATAGAGTC

Figure 27 AK

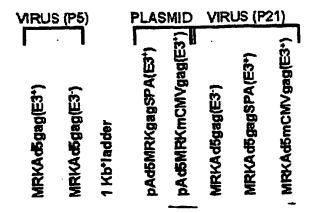
			AATAGGCCAT		
35101	CCGGTAAGAC GGCCATTCTG	ACGACTTATC TGCTGAATAG	GCCACTGGCA CGGTGACCGT	GCAGCCACTG CGTCGGTGAC	GTAACAGGAT CATTGTCCTA
35151	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC
33131	ATCGTCTCGC	TCCATACATC	CGCCACGATG	TCTCAAGAAC	TTCACCACCG
35201	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG
,33201	GATTGATGCC	GATGTGATCT	TCCTGTCATA	AACCATAGAC	GCGAGACGAC
35251	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA
			TTCTCAACCA		
35301	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG	CAGATTACGC
			CAAAAAAACA		
35351	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT
			CTTCTAGGAA		
35401	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT
			GAGTGCAATT		
35451	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATCAATCT	AAAGTATATA
			TCTAGGAAAA		
35501	TGAGTAAACT	TGGTCTGACA	GTTACCAATG	CTTAATCAGT	GAGGCACCTA
		•			CTCCGTGGAT
35551	TCTCAGCGAT	CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC
					TGAGGGGCAG
35601	GTGTAGATAA	CTACGATACG	GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC
					GGTCACGACG
35651	AATGATACCG	CGAGACCCAC	GCTCACCGGC	TCCAGATTTA	TCAGCAATAA
					AGTCGTTATT
35701	ACCAGCCAGC	CGGAAGGGCC	GAGCGCAGAA	GTGGTCCTGC	AACTTTATCC
					TTGAAATAGG
35751	GCCTCCATCC	AGTCTATTAA	TTGTTGCCGG	GAAGCTAGAG	TAAGTAGTTC
				•	ATTCATCAAG
35801	GCCAGTTAAT	AGTTTGCGCA	ACGTTGTTGC	CATTGCTACA	GGCATCGTGG
					CCGTAGCACC
35851	TGTCACGCTC	GTCGTTTGGT	ATGGCTTCAT	TCAGCTCCGG	TTCCCAACGA
					AAGGGTTGCT
35901	TCAAGGCGAG	TTACATGATO	CCCCATGTTG	TGCAAAAAA	COCANTICAC
					GCCAATCGAG
35951	CTTCGGTCCT	CCGATCGTTC	TCAGAAGTAA	GTTGGCCGC	A GTGTTATCAC
	GAAGCCAGGA	GGCTAGCAAC	AGTCTTCATT	CAACCGGCG	CACAATAGTG



36051		CTGTGACTGG			
		GACACTGACC			
36101	GTGTATGCGG	CGACCGAGTT	GCTCTTGCCC	GGCGTCAACA	CGGGATAATA
	CACATACGCC	GCTGGCTCAA	CGAGAACGGG	CCGCAGTTGT	GCCCTATTAT
36151		TAGCAGAACT			
		ATCGTCTTGA			
36201		AACTCTCAAG			
		TTGAGAGTTC			
36251		CGTGCACCCA			
		GCACGTGGGT			
36301		GTGAGCAAAA			
		CACTCGTTTT	•		
36351	ATAAGGGCGA				
		GTGCCTTTAC			
36401		ATTTATCAGG			
		TAAATAGTCC			
36451	AATGTATTTA				
		CTTTTTATTT			
36501	AAAGTGCCAC				
		GACTGCAGAT			
36551	TAAAAATAGG				
	ATTTTTATCC	GCATAGTGCT	CCGGGAAAGC	AGAAGTTCTT	AACCTAGGCT
		PacI			
36601	ATTCTTAATT	TCTTAATTAA	(SEQ ID NO:	34)	

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34) TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Tigure 27 AM



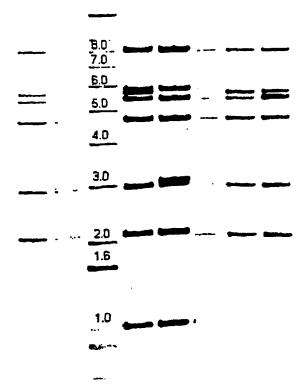


FIGURE 28

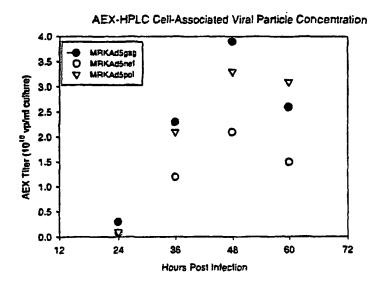


FIGURE 29A

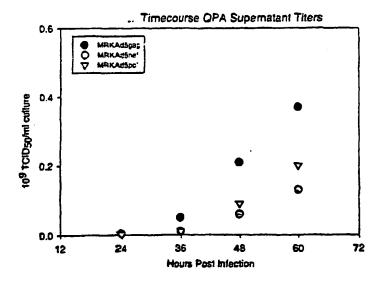


FIGURE 29B

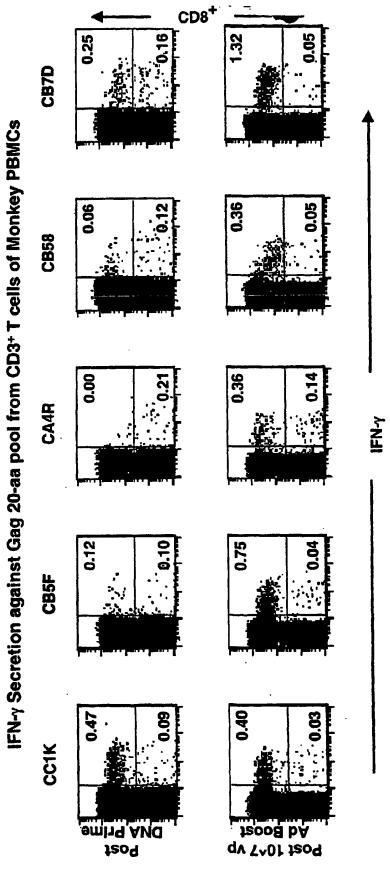
atg Met 1	gat Asp	gca Ala	atg Met	aag Lys 5	aga Arg	GJY ggg	ctc Leu	tgc Cys	tgt Cys 10	gtg Val	ctg Leu	ctg Leu	ctg Leu	tgt Cys 15	Gly gga	48
gca Ala	gtc Val	ttc Phe	gtt Val 20	tcg Ser	ccc Pro	agc Ser	gag Glu	atc Ile 25	tcc Ser	att Ile	gtg Val	tgg Trp	gcc Ala 30	tcc Ser	agg Arg	96
gag Glu	ctg Leu	gag Glu 35	agg Arg	ttt Phe	gct Ala	gtg Val	aac Asn 40	cct Pro	ggc Gly	ctg Leu	ctg Leu	gag Glu 45	acc Thr	tct Ser	gag Glu	144
Gly ggg	tgc Cys 50	agg Arg	cag Gln	atc Ile	ctg Leu	ggc Gly 55	cag Gln	ctc Leu	cag Gln	ecc Pro	tcc Ser 60	ctg Leu	caa Gln	aca Thr	Gly ggc	192
tct Ser 65	gag Glu	gag Glu	ctg Leu	agg	tcc Ser 70	ctg Leu	tac Tyr	aac Asn	aca Thr	gtg Val 75	gct Ala	acc Thr	ctg Leu	tac Tyr	tgt Cys 80	240
gtg Val	cac His	cag Gln	aag Lys	att Ile 85	gat Asp	gtg Val	aag Lys	gac	acc Thr 90	aag Lys	gag Glu	gcc Ala	ctg Leu	gag Glu 95	aag Lys	288
att Ile	gag Glu	gag Glu	gag Glu 100	cag Gln	aac Asn	aag Lys	tcc Ser	aag Lys 105	Γλ2	aag Lys	gcc Ala	cag Gln	cag Gln 110	gct Ala	gct Ala	336
gct Ala	ggc Gly	aca Thr 115	Gly	aac Asn	tcc Ser	agc Ser	cag Gln 120	vaı	tcc Ser	cag Gln	aac Asn	tac Tyr 125	120	att	gtg Val	384
cag Gln	aac Asn 130	Leu	cag	Gly	cag Gln	atg Met 135	gtg Val	Cac	cag Gln	gcc Ala	atc Ile 140	Ser	ccc Pro	cgg	acc Thr	432
ctg Lev 145	a Asn	gcc	tgg Trp	gtg Val	aag Lys 150	var	gtg Val	gag Glu	g gag n Glu	aag Lys 155	. WTG	tto Phe	tcc Ser	Pro	gag Glu 160	480
gtg Val	ato Ile	ccc Pro	atg Met	ttc Phe 165	Ser	gcc	ctg Lev	tct Sea	gag Glv 170	ניטי	gcc Ala	aco Thi	e ccc	Gl: 17	gac Asp	528
ctç Lei	g aac u Asr	aco Thi	ato Met	Let	aac Asr	aca Thr	gto Val	ggg L Gl 18	Ā GT7	cat His	cag Gl	g gct	gco Ala 190		g cag t Gln	576
at (g cto t Lei	aaq Lys 195	s Gli	aco Thi	ato Ile	aat Asr	gag Gl: 200	7 67	g gct u Ala	get Ala	t gag a Gli	tg Tr 20	P1	ag Ar	g ctg g Lev	624
ca [*]	t cci s Pro 21	va.	g cad	gct Ala	gg(a Gly	7 Pro 21	5 770	t gc e Al	e cco	gge Gl	y Gl: 22	n Me	g ag	g ga g Gl	g ccc	672
Ar 22	g Gl; 5	y Se:	r Asj) II(23	0	y TI	r in	1 26.	23	5				g att n Ile 240	
G1	c tg y Tr	g at p Me	g ac	c aac r As: 24	n As	c ec	c cc o Pr	c at o Il	c cc e Pr 25	Ų VI	g gg 1 Gl	y Gl	a at u Il	c ta e Ty 25	r Ly: 55	g 768 s

Figure 30'A°

agg Arg	tgg Trp	atc Ile	atc Ile 260	Leu	ggc	ctg Leu	aac Asn	aag Lys 265	att Ile	gtg Val	agg A rg	atg Met	tac Tyr 270	tcc Ser	ecc Pro	816
			Leu				cag Gln 280									864
		Asp					acc Thr									912
							gag Glu									960
							aag Lys									1008
							cag Gln									1056
gcc	agg Arg	gtg Val 355	ctg Leu	gct Ala	gag Glu	gcc Ala	atg Met 360	tcc Ser	cag Gln	gtg Val	acc Thr	aac Asn 365	tcc Ser	gcc Ala	acc Thr	1104
							ttc Phe									1152
	Phe						ggc									1200
							aag Lys									1248
aag Lys	gac Asp	tgc Cys	aat Asn 420	gag Glu	agg Arg	cag Gln	gcc Ala	aac Asn 425	ttc Phe	ctg Leu	C ly ggc	aaa Lys	atc Ile 430	tgg Trp	Pro	1296
							aac Asn 440									1344
							ttc Phe									1392
ccc Pro 465	agc Ser	cag Gln	aag Lys	cag Gln	gag Glu 470	ccc Pro	att Ile	gac Asp	aag Lys	gag Glu 475	ctg Leu	tac Tyr	ccc Pro	ctg Leu	gcc Ala 480	1440
tcc Ser	ctg Leu	agg Arg	tcc Ser	ctg Leu 485	ttt Phe	ggc	aac Asn	gac Asp	ccc Pro 490	tcc Ser	tcc Ser	cag Gln	taa	(SII	NO:36) NO:37)	1482

Figure 30 B

Figure 31



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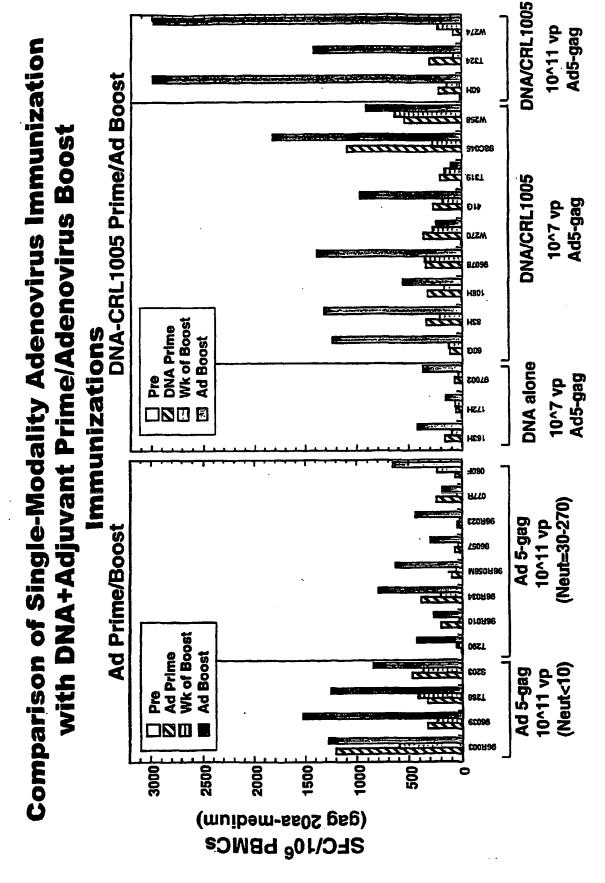


FIGURE 33A

	GGGCTTCTGT				
	GTGGCAAGAA		•		
	TTGCTGTGAA				
CTGGGCCAGC	TCCAGCCCTC	CCTGCAAACA	GGCTCTGAGG	AGCTGAGGTC	CCTGTACAAC
ACAGTGGCTA	CCCTGTACTG	TGTGCACCAG	AAGATTGATG	TGAAGGACAC	CAAGGAGGCC
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	CCCTGTCTGA	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	GTGGGGGGCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
GGCCCCATTG	CCCCGGCCA	GATGAGGGAG	CCCAGGGGCT	CTGACATTGC	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	TGGCTGGATG	ACCAACAACC	CCCCATCCC	TGTGGGGGAA
ATCTACAAGA	GGTGGATCAT	CCTGGGCCTG	AACAAGATTG	TGAGGATGTA	CTCCCCCACC
TCCATCCTGG	ACATCAGGCA	GGGCCCCAAG	GAGCCCTTCA	GGGACTATGT	GGACAGGTTC
TACAAGACCC	TGAGGGCTGA	GCAGGCCTCC	CAGGAGGTGA	AGAACTGGAT	GACAGAGACC
CTGCTGGTGC	AGAATGCCAA	CCCTGACTGC	AAGACCATCC	TGAAGGCCCT	GGGCCCTGCT
GCCACCCTGG	AGGAGATGAT	GACAGCCTGC	CAGGGGGTGG	GGGGCCCTGG	TCACAAGGCC
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
GGCAACTTCA	GGAACCAGAG	GAAGACAGTG	AAGTGCTTCA	ACTGTGGCAA	GGTGGGCCAC
ATTGCCAAGA	ACTGTAGGGC	CCCCAGGAAG	AAGGGCTGCT	GGAAGTGTGG	CAAGGAGGC
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	TGGGCAAAAT	CTGGCCCTCC
CACAAGGGCA	GGCCTGGCAA	CTTCCTCCAG	TCCAGGCCTG	AGCCCACAGC	CCCTCCCGAG
GAGTCCTTCA	GGTTTGGGGA	GGAGAAGACC	ACCCCCAGCC	AGAAGCAGGA	GCCCATTGAC
AAGGAGCTGT	ACCCCCTGGC	CTCCCTGAGG	TCCCTGTTTG	GCAACGACCC	CTCCTCCCAG
					CATGGATGGC
CCCAAGGTGA	AGCAGTGGCC	CCTGACTGAG	GAGAAGATCA	AGGCCCTGGT	GGAAATCTGC
	AGAAGGAGGG				
					GGACTTCAGG
					CCACCCCGCT
					CTTCTCTGTG
					CAACAATGAG
					CTCCCCTGCC
					CCCTGACATT
					TGGGCAGCAC
					CACCCCTGAC
					CCCCGACAAG
					TGACATCCAG
					GGTGAGGCAG
					GACTGAGGAG
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG	AGCCTGTGC	A TGGGGTGTAC

FIGURE 33B

				AGGGCCAGGG	
				GCAAGTATGC	
GGGGCCCACA	CCAATGATGT	GAAGCAGCTG	ACTGAGGCTG	TGCAGAAGAT	CACCACTGAG
TCCATTGTGA	TCTGGGGCAA	GACCCCCAAG	TTCAAGCTGC	CCATCCAGAA	GGAGACCTGG
GAGACCTGGT	GGACTGAGTA	CTGGCAGGCC	ACCTGGATCC	CTGAGTGGGA	GTTTGTGAAC
ACCCCCCCC	TGGTGAAGCT	GTGGTACCAG.	CTGGAGAAGG	AGCCCATTGT	GGGGGCTGAG
ACCTTCTATG	TGGCTGGGGC	TGCCAACAGG	GAGACCAAGC	TGGGCAAGGC	TGGCTATGTG
ACCAACAGGG	GCAGGCAGAA	GGTGGTGACC	CTGACTGACA	CCACCAACCA	GAAGACTGCC
CTCCAGGCCA	TCTACCTGGC	CCTCCAGGAC	TCTGGCCTGG	AGGTGAACAT	TGTGACTGCC
TCCCAGTATG	CCCTGGGCAT	CATCCAGGCC	CAGCCTGATC	AGTCTGAGTC	TGAGCTGGTG
AACCAGATCA	TTGAGCAGCT	GATCAAGAAG	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC
CACAAGGGCA	TTGGGGGCAA	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG
GTGCTGTTCC	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCTGTGG	TGGCTAAGGA	GATTGTGGCC
TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG	GCCATGCATG	GGCAGGTGGA	CTGCTCCCCT
GGCATCTGGC	AGCTGGCCTG	CACCCACCTG	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT
GTGGCCTCCG	GCTACATTGA	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC
TACTTCCTGC	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT	CAAGCAGGAG
TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG	CCTCCATGAA	CAAGGAGCTG
AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG
GCTGTGTTCA	TCCACAACTT	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG
AGGATTGTGG	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG	GAAGGGCCCT
GCCAAGCTGC	TGTGGAAGGG	GGAGGGGGCT	GTGGTGATCC	AGGACAACTC	TGACATCAAG
GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC	AGGGACTATG	GCAAGCAGAT	GGCTGGGGAT
GACTGTGTGG	CCTCCAGGCA	GGATGAGGAC	TAA		
SEQ ID NO:	38				

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Jle Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

International application No.

PCT/US01/28861

A. CLAS	SIFICATION OF SUBJECT MATTER : C12N 15/86			
US CL	: 435/456		1	
	International Patent Classification (IPC) or to both nat	tional classification and IPC		
	DS SEARCHED			
	cumentation searched (classification system followed by 24/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3,			
Documentation	on searched other than minimum documentation to the	extent that such documents are included	i in the fields searched	
	ta base consulted during the international search (name ontinuation Sheet	of data base and, where practicable, s	earch terms used)	
C. DOCI	JMENTS CONSIDERED TO BE RELEVANT			
Category *	Citation of document, with indication, where app	ropriate, of the relevant passages	Relevant to claim No.	
Х	WO 96/39178 (ERTL et al.) 12 December 1996 (12.1	12.1996), see page 5, 6,10, 12, 13	1-3, 8-11, 18	
Ÿ	and claims 1 and 5.		4, 5, 13-17, 29-32, 34, 35, 37	
x	US 6,019,978 A (ERTL et al.) 1 February 2000 (01/0	02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18	
Y Y			4, 5, 13-17, 29-32, 34, 35, 37	
X,P	US 6,287,571 b (ERTL et al.) 11 September 2001 and claim 1.	(11/09/2001), see columns 2, 7, 8	1, 9, 18	
x	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.			
Y	4,5,13-17, 29-32, 34, 35, 37			
Y	WANG et al. The use of an E1-deleted, replication - expressing the rabies virus glycoprotein for early vac Journal of Virology (March 1997) Vol. 71, No. 5, pp	cination of mice against rabies virus.	1-3, 9-11, 13-18	
D Earthor	documents are listed in the continuation of Box C.	See patent family annex.	1	
<u> </u>		"T" later document published after the in	sternational filing date or	
 Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance 		priority date and not in conflict with understand the principle or theory u	the application but cited to aderlying the invention	
"E" earlier ap	plication or patent published on or after the international filing	 X° document of particular relevance; the considered novel or cannot be considered novel or cannot be considered novel or cannot be considered when the document is taken all 	dered to involve an inventive	
	t which may throw doubts on priority claim(s) or which is cited sh the publication date of another citation or other special reason (led)	"Y" document of particular relevance; the considered to involve an inventive a combined with one or more other as combination being obvious to a per	tep when the document is selt documents, such	
"O" document referring to an oral disclosure, use, exhibition or other means "&" document member of the same patent family				
P document published prior to the international filing date but later than the priority date claimed				
Date of the actual completion of the international search Of February 2002 (06.02.2002) Date of mailing of the international search report 19 AUG 2002				
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Con Box	missioner of Patents and Trademarks PCT	Ulrike Winkler, Ph.D.	alblins fo	
	chington, D.C. 20231 b. (703)305-3230	Telephone No. 703-308-0196	1)	
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INTERNATIONAL SEARCH REPORT

C. (Contin	uation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category •	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

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This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons: Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely: Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a). Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet) This international Searching Authority found multiple inventions in this international application, as follows: Please Sec Continuation Sheet As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. As all required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37 Remark on Protest	Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)				
because they relate to subject matter not required to be searched by this Authority, namely: 2.	This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:				
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: 3.					
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a). Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet) This international Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet 1.	because they relate to parts of the international application that do not comply with the prescribed requirements to				
This International Searching Authority found multiple inventions in this international application, as follows: 1.	because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule				
Please See Continuation Sheet 1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. 2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37 Remark on Protest The additional search fees were accompanied by the applicant's protest.	Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)	ļ			
searchable claims. 2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37 Remark on Protest The additional search fees were accompanied by the applicant's protest.					
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	is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 35, 37	яt 34,			

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> and <u>AE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of Ε1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$

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	and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
T	I take the contains the circucting peckeding sequence of the wild type
	and AE3, the vector contains the tis-acting packaging selection and the selection contains the tis-acting packaging selection and the selection contains the tis-acting packaging selection and the selection contains the tis-acting packaging selection and the selection contains the tis-acting packaging selection and the selection contains the tis-acting packaging selection and the selection contains the tis-acting packaging selection and the selection contains the selection conta
1	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
1	inserted in E1.
55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
	and AE3, the vector contains the cis-acting packaging sequence of the wild type
1	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
	inserted in E1.
55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
	and AE3, the vector contains the cis-acting packaging sequence of the wild type
:	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
	inserted in E1.
57-61	The claims are directed to a method of making and harvesting of a recombinant
<u> </u>	adenoviral particle that contains a gene encoding an HIV Pol protein.
62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
	to HIV Pol protein with the recombinant adenoviral particle.
63, 64	The claim is directed to a method of generating a cellular mediated immune response
	to HIV Pol protein with the recombinant adenoviral particle in addition to
<u> </u>	administering a DNA plasmid vaccine.
	The claims are directed to an adenoviral vector that is at least partially deleted of
73, 75	ΔEI, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
1	adenovirus genome, and a gene which checkes all rity her process (SEC)
	inserted in the parallel orientation of E1. The claims are directed to an adenoviral vector that is at least partially deleted of
1	The claims are directed to an adenoviral vector that is at least partially directed of ΔE_1 , the vector contains the cis-acting packaging sequence of the wild type
73, 75	$\Delta E1$, the vector contains the cis-acting packaging sequence of the wind type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	adenovirus genome, and a gene which encodes an invited proum (022 22 10 10)
	inserted in the parallel orientation of E1. The claims are directed to an adenoviral vector that is at least partially deleted of
	The claims are directed to an ademoviral vector that is at least partially decreased and the wild type
73, 75	<u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
	inserted in the parallel orientation of E1.
	The claims are directed to an adenoviral vector that is at least partially deleted of
1	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type
73,75	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
	inserted in the parallel orientation of E1.
 	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$.
71	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
1	the antiparallel orientation of E1.
 	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$.
/1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
	the antiparallel orientation of E1.
- 21	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI .
//	the amotor contains the cis-acting nackaging sequence of the wild type adenovirus
1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
1	the antiparallel orientation of E1.
 	The claim is directed to an adenoviral vector that is at least partially deleted of AEI.
1"	the spaces contains the dispecting packaging sequence of the wild type adenovirus
1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
	the entire relief orientation of E1.
74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE
/4	and AE3 the vector contains the cis-acting packaging sequence of the wild type
Ì	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
ŀ	inserted in El
 	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE
74	and AE3 the vector contains the cis-acting packaging sequence of the wild type
1	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	inserted in E1.
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74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI
	55 57-61 62, 65, 66 63, 64 67-70, 72,

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		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type
	1	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune
		response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune
		response to HIV Nef with the recombinant adenoviral particle in addition to
		administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed
_		from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
	i	from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are trawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from two individual vectors, one expressing gag-pol fusion and one expressing nef.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
	Ì	from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
	1	from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
•	1	individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
	30., 50	from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
7.7	003, 00, 05	from individually from one vector.
44	86k. 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
	50x, 55	individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
43	301, 50, 65	individually from one vector.
46	86m. 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a
40	90III, 86	fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as a
4/	90II, 69	•
40	96- 99	fusion protein from one vector.
48	860, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a
		fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5. 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter